Anatomy of the Persisylvian language pathways in the living human brian A diffusion tensor imaging approach.

Budisavljevic, Sanja

Awarding institution:
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Author: Sanja Budisavljevic

Anatomy of the Perisylvian Language Pathways in the Living Human Brain

A Diffusion Tensor Imaging Approach

Sanja Budisavljević

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The research on language in the brain, this most eloquent “ghost in the machine”, has enkindled my imagination since the early teenage years and has been my passion ever since. This thesis began as a question hidden in my grandfather’s library smelling of wild pomegranates, my father’s medical books that I secretly read and admired, and largely forgotten Soviet publications in the midsts of war-struck Yugoslavia. However, the thesis would not have been completed without the help and support of many people. Although they did not type, write or edit my words, their presence is felt in every syllable of this thesis.

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I will be forever grateful to my dearest friends who have been my support and my finest source of smiles and inspiration on this journey. I extend my deepest gratitude to Marko who endured my long working hours and brought me sunshine in the breaks - without him this would be a lonesome adventure. I want to thank my family without whom I would not be here today, especially my grandmother for her warmest smiles and love that coloured my childhood; my grandfather for igniting in me the passion for words and intellectual thought that inspired me to take this route; my best brother in the world who supported me and believed in me throughout this journey. Most of all, I am eternally grateful and proud of my beloved parents, who have been my shoulders to stand on, my biggest support, strength and inspiration, and who thought me the most important thing - to follow my dreams and believe in myself. I dedicate this thesis to them.

Abstract

Language is a unique human ability influenced by genetic, cultural and social factors. Decades of research in the field have identified those brain networks that have made the development of language in humans possible. This PhD thesis introduces three studies that aim to explore the anatomy of the perisylvian language pathways (three segments of the *arcuate fasciculus*) both in healthy and pathological condition, using dffusion tensor imaging tractography. First study examined typical developmental trajectories of the perisylvian language network in a normative data of 101 subjects (age range: 9-49 years). After observing how these pathways mature across life span, second study investigated how these are influenced by genes and environment in a sample of 43 adult twin pairs (26 monozygotic and 17 dizygotic pairs). The results showed that perisylvian language pathways exhibit distinct maturational patterns and vary in respect to genetic control that guides this process. Familial effects played an important role for those tracts that lateralised early in life (frontal lobe connections), whereas those tracts that continue to remodel throughout adolescence (temporo-parietal connections) were driven more by unique environmental effects. While the first two studies explored anatomy of perisylvian language pathways in healthy population, third study examined neural correlates in a pathological condition that affects language processing. This study included 61 adults with autism spectrum disorder and 61 matched neurotypical controls. Localised abnormalities were identified in the left perisylvian language pathways in people with autism spectrum disorder and an association was found between these white matter abnormalities and severity of past language deficits. In conclusion, these findings may be important to furthering our knowledge of the anatomy of the perisylvian language pathways in healthy population. Also, they may facilitate our understanding of possible biological mechanisms that underpin language dysfunction in psychiatric disorders, and lead to new approaches for early diagnosis and treatment.

Outlines of the Thesis

The PhD thesis is broken up into five major sections describing three diffusion tractography studies (for a graphical representation see the following page). The first part of the thesis - **Chapter 1** - offers an introduction to the perisylvian language pathways in the human brain and diffusion tensor imaging tractography. The second part of the thesis - **Chapter 2** -describes the first diffusion tractography study on the maturation of the perisylvian language pathways using healthy cross-sectional data of children, adolescents and adults. The third part of the thesis tackles the questions of heritability of the perisylvian language network, and is divided into following two Chapters. **Chapter 3** is an introduction to imaging genetics and classical twin design used in the second tractography study, discussed in Chapter 4. **Chapter 4** investigates the nature-nurture debate in relation to the anatomy of the perisylvian language pathways as described by diffusion tensor imaging tractography. The fourth part of the thesis - Chapter 5 and 6 - introduces the third diffusion tractography study investigating perisylvian language pathways in autism. **Chapter 5** is a general introduction to autism spectrum disorders. **Chapter 6** describes the third diffusion tractography study investigating neuroanatomical and neuropsychological data on language impairment in autism spectrum disorders. It explores how impaired language development is associated with abnormalities in the perisylvian language pathways known to support language processes in the healthy adult brain. Finally, the fifth part of the thesis - **Chapter 7** - briefly summarises the results of the mentioned studies and provides final conclusions.

ANATOMY
of the
PERISYLVIAN LANGUAGE PATHWAYS

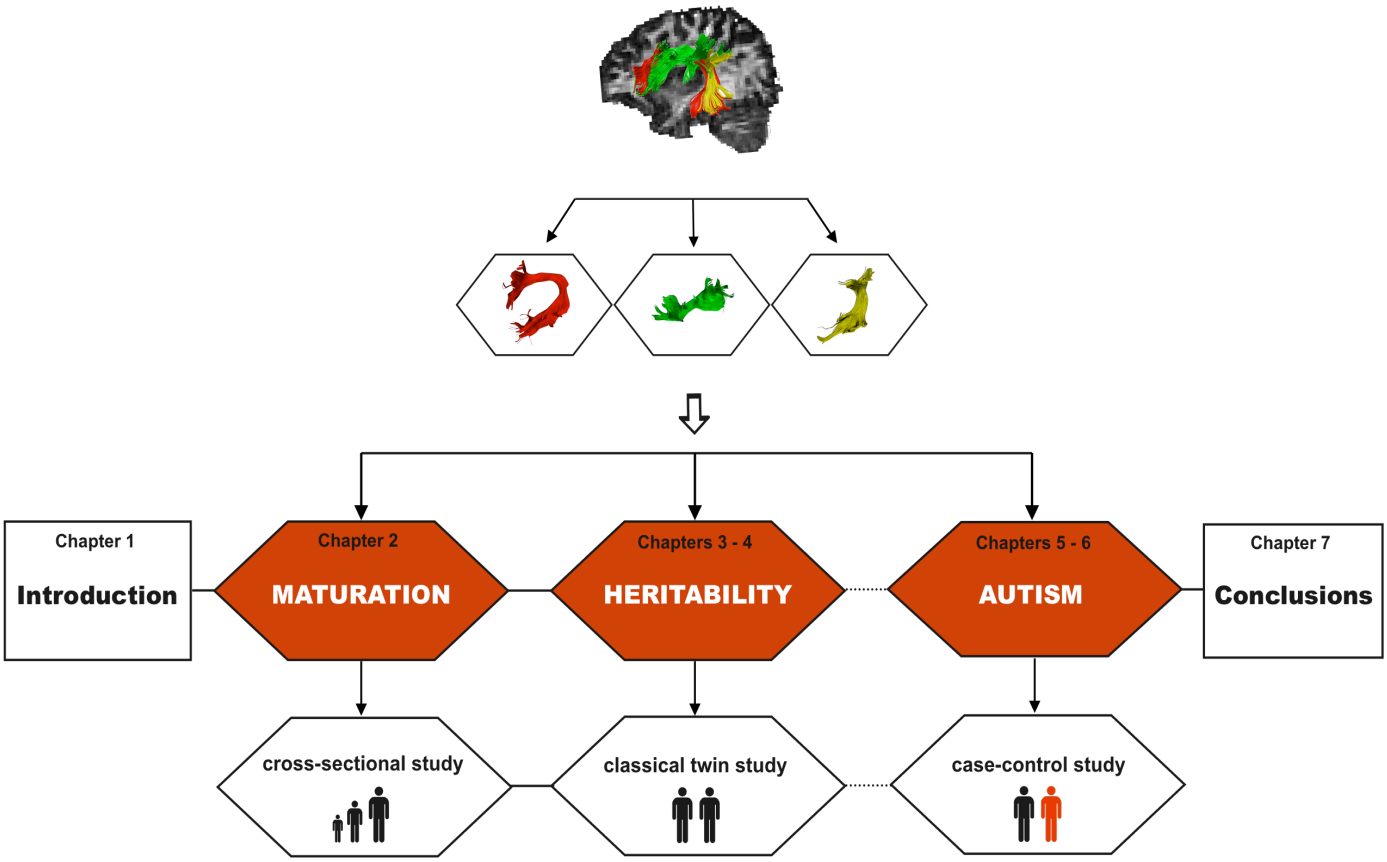

Table of Contents

Ch. 3. Imaging Genetics and Twin Methodology

Ch. 4. Heritability of the Perisylvian Language Pathways

Chapter 5. Introduction to Autism Spectrum Disorder

Chapter 6. Perisylvian Language Pathways in Autism Spectrum Disorders

List of Figures

Chapter 1

Chapter 2

Chapter 3

Chapter 4

Chapter 6

Figure 6.3.1 Tractography reconstructions of the long (in red), anterior (in green) and posterior (in yellow) segments of the perisylvian language network from the healthy subject (on the left) and ASD subject (on the right).

Figure 6.3.2 Overview of the significant differences (at $p < 0.05$) found in the ASD group compared to controls, in FA - fractional anisotropy, MD - mean diffusivity, Dperp - perpendicular diffusivity, NoSt - number of streamlines, Vol - volume; n.s.- not significant, , * significant at $p < 0.05$; *** significant at $p < 0.01$

Figure 6.3.3 Histogram of the mean number of streamlines (±95% confidence interval) in the left anterior segment for the three subgroups of ASD patients divided according to the severity of stereotyped utterances and delayed echolalia (for ADI-R question 33, score 0 corresponds to normal, 1 to mild symptoms, 2 to severe symptoms). (* p=0.035 between normal and mild, after Bonferroni correction; ** p=0.027 between normal and severe, after Bonferroni correction).

List of Tables

Chapter 1

Chapter 2

Chapter 4

Chapter 6

Chapter 1

Introduction to the perisylvian language pathways: brain and language

In the last decade tractography methods based on diffusion imaging have rekindled an interest in the neuroanatomical basis of language. This chapter is devoted to the harmonisation of the neuroanatomical findings from post-mortem dissection to more recent evidence emerging from diffusion tractography. The chapter will explore basic concepts linked to the anatomy of the perisylvian language pathways (i.e. the arcuate fasciculus), while placing them in a wider historical and evolutionary context. Further, it will discuss the asymmetry of the arcuate fasciculus, its heterogeneity in the normal population, and possible functional and behavioural correlates. The chapter also provides an introduction to diffusion tensor imaging tractography, a method used in this PhD study.

1.1 The cerebral organisation of language: historical overview

The earliest known written document on the loss of speech is Edwin Smith's Surgical Papyrus from ancient Egypt (1600 BC) (Wilkins, 1992), which is itself based on an even earlier document. Hence, it could be speculated that humans have been investigating language for circa 4000 years. However, the brain as a possible culprit for the loss of speech remained unstudied for most of that time. Even in Greco-Roman times tongue paralysis and not the brain was regarded as the source of all speech disorders. The first time that the brain was given due importance was during the Dark and Middle Ages, when Church Fathers, influential theologians, claimed that the fourth ventricle of the brain was responsible for aphasia (loss of speech) syndromes, believing it to be the seat of memory for words. Renaissance brought further approval that speech disorders were due to excessive phlegm in the fourth ventricle, with Paracelsus noting that speech disturbances can occur with or without tongue paralysis (Finger, 2001).

It was in the 19th century that speech and language were localised in the cortex, based upon phrenological beliefs that the cerebral cortex is subdivided into functional units. Phrenologist Franz Joseph Gall placed the faculty of language in the frontal lobes, and although coming close to what is known today about the importance of the frontal lobes for language, his deductions were merely speculations. Gall gives an example of his classmate with large bulging eyes who had an exceptional memory for words, and explains that his talent was due to the abundance of frontal lobe brain tissue protruding to push his eyes forward (Finger, 2001). Although Gall's deductions were unsupported by facts, he deserves the credit for pioneering cerebral localisation of language function and surmising the importance of the frontal lobes. Subsequently, French physician Jean-Baptiste Bouillaud supported Gall's hypothesis about frontal localisation of speech, basing his conclusion upon 500 cases with speech pathology affecting the frontal lobes of the brain. Bouillaud was the first to systematically study language localisation in a bigger sample, and could be regarded as the first statistician in the history of neuroscience.

Analysing deficits resulting from lesions (localised damage) due to illness or injury, like Bouillaud did, is one of the oldest approaches to identifying the function of specific brain region (Bechtel, 2004). Upon the death of the patient, a correlation between the symptoms observed during life and the loss of brain substance found at post-mortem examination enabled neurologists to associate specific parts of the brain surface with certain functions (Mott, 1910). The classical example of using brain lesions to refine our knowledge on how the brain processes language was Paul Broca's (1824-1880) study of two patients, Leborgne and Lelong. When Broca first encountered a 51-year old Leborgne in his surgical service in April 1861, he had been hospitalized for circa 20 years (Bechtel, 2004). Other patients called him *Tan* because this sound and few other words were his only utterances. Similarly, patient Lelong was 84-year old labourer who was able to say only a few simple words (e.g. oui, non, trois, toujours) and could not write. After Leborgne and Lelong died, Broca performed an autopsy and revealed that both patients had a lesion localised in the third frontal convolution of the left hemisphere (see Fig 1.1.1). Following this, Broca gathered similar evidence from a number of other patients and maintained that the inability to speak was limited to this frontal area. Subsequently the third frontal convolution, known as *Broca's area,* became known as the locus of articulate speech.

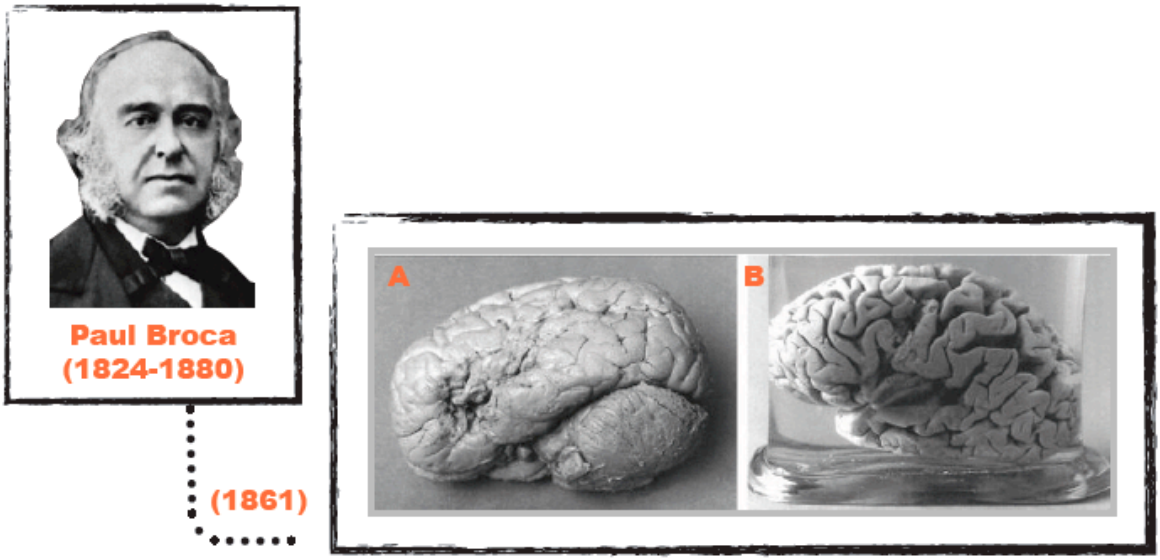

Fig 1.1.1 Photographs showing lateral views of the brains of *Leborgne* (A) and *Lelong* (B) adapted from Dronkers et al. (2007). Paul Broca preserved the organs by immersing them in alcohol; he then donated them to the Musée Dupuytren in Paris.

Not everyone agreed with Broca's findings. Neurologist Pierre Marie published a paper in 1906 entitled "The third frontal convolution plays no special role whatsoever in the function of language" and stated that none of the older observations of Broca can be accepted because of the methodological limitations and inability to reveal the full extent of brain damage (Mott, 1910). A decade after Broca's work, Carl Wernicke (1874) described a different pattern of language deficit, affecting comprehension of language following lesions in a part of the temporal cortex known today as *Wernicke's area* (Bechtel, 2004). This clinico-anatomical correlation method encountered fierce opposition with the rise of holistic ideas during the first half of the 20th century, which started a famous holistic-localisationist controversy. John Hughlings Jackson, Constantin von Monakow, Henry Head, Karl Lashley and Kurt Goldstein warned that to locate the damage which destroys speech and to locate speech are two different things - localisation of symptoms is different from localisation of function. They believed that symptoms can arise also due to secondary and distant 'hodological' effects. The tradition of Broca and Wernicke was revived later in the 1960s in a series of influential papers by Norman Geschwind, who added new insights to the brain connectivity underlying language function. Geschwind brought new credibility to the localisationist approach by re-interpreting the functional role of connections and specialized cortical areas according to evidence arising from the new methodologies that became available during the 20th Century (Catani and Mesulam, 2008).

Before blunt dissection methodology appeared in the 19th century, there was a lack of scientific information on human connectional neuroanatomy. The dissection techniques for white matter tracts developed by early neuroanatomists led to important anatomical discoveries about the connection pathways. Much of the current understanding of the anatomy of the perisylvian language pathways –i.e. the arcuate fasciculus - which will be explored in this PhD thesis, is based on the work of 19th century neuroanatomists, such as Johann Christian Reil, Karl Friedrich Burdach, Theodor Hermann Meynert, Carl Wernicke, Ludwig Lichtheim, and Jules Dejerine (see Fig 1.1.2).

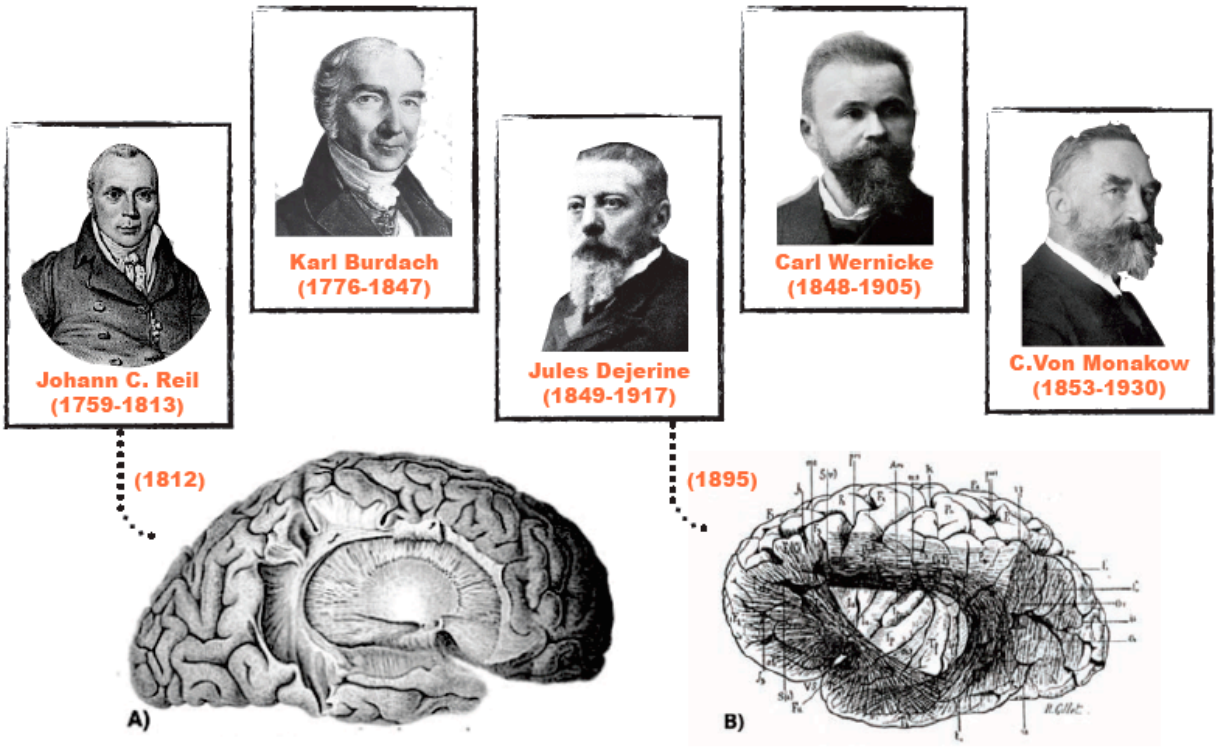

Fig 1.1.2 Some of the major 19th century neuroanatomists who have contributed to our understanding of the anatomy of the arcuate fasciculus; A) Reil's (1812) and B) Dejerine's (1895) post-mortem dissections of the arcuate fasciculus.

The arcuate fasciculus, a large association tract connecting perisylvian areas of the frontal, temporal and parietal lobes of each hemisphere was first described at the beginning of the 19th century by Johann Christian Reil (1809, 1812). Reil developed a dissection method whereby he soaked the brain in alcohol to make it more suitable for dissecting white matter bundles. Upon his discovery of the arcuate fasciculus arching around the Sylvian fissure of the right hemisphere, he described it as *the unnamed white matter substance* (i.e., *Ungenannte Marksubstanz* (Catani, et al., 2010)). Reil's findings were confirmed a decade later by Karl Friedrich Burdach (1822), who was the first to use Latin name *Fasciculus Arcuatus* (arcuate fasciculus) due to its arching shape. This name became widely accepted and remains unchanged in the current international nomenclature. Subsequently, Jules Dejerine (1895) confirmed the findings of the two German neuroanatomists, but attributed the discovery to Burdach. Dejerine further believed that the arcuate fasciculus was mainly composed of short associative fibres connecting neighbouring perisylvian cortex (Catani, 2009). Burdach and Dejerine considered the arcuate fasciculus to be part of the superior longitudinal fasciculus and used these two terms interchangeably in their descriptions (Martino, et al., 2012). For almost 90 years since its first description, the functions of the arcuate fasciculus remained unknown and no association was made to language. The first scientist who attributed a role in language processing indirectly to the arcuate fasciculus was Carl Wernicke, who postulated that language relies on the integrity of a "psychic reflex arc" between temporal and frontal regions (Catani, 2009). However, the arcuate fasciculus was not part of Wernicke's original anatomical model as he thought that the temporal and frontal language

areas were mutually interconnected by fibres passing through the external capsule and relaying in the insular cortex (Wernicke, 1874). Constatin von Monakow was the first to identify the arcuate fasciculus as the tract connecting Broca's and Wernicke's areas in 1897, a view later accepted by Wernicke in 1908 (Geschwind, 1967).

This view, which only in part overlapped with more complex but anatomically unproven models of language (e.g. Lichtheim's house (Lichtheim, 1887)), attracted initially the favour of many aphasiologists. However, others, like Pierre Marie, criticised this model and drew attention to subcortical structures involved in language disorders. This approach culminated in the work of Penfield, who in 1959 co-authored with Roberts a monograph on language, where emphasis was placed on thalamo-cortical connections rather than cortico-cortical pathways. The arcuate fasciculus model was later revitalised by Norman Geschwind who had the merit of including the inferior parietal region among other language areas. Geschwind's model was in part supported by early neuroimaging studies based on radioisotope brain scanning and computerised tomography that permitted to refine the cortical localisation of the major cortical syndromes. Nevertheless, the lack of advanced methods for studying connections in the human brain forced neurologists to rely entirely on animal models. This approach had two inherent problems. First it did not allow to test whether tracts identified in monkeys have a role in language. Second it implied that tracts serving language functions are preserved along the phylogeny scale, which is rather speculative considering that animals do not have language.

Recent advances in diffusion imaging have rekindled an interest in language related brain research, and together with the studies of aphasics over the last century, refined the knowledge of how the brain processes language. The existence of the arcuate fasciculus was confirmed in human post-mortem studies using different methods like blunt dissections, and axonal staining of degenerating axons. However, dissection methods are inadequate for quantitative studies of this tract and hence have not shed much light on the characteristics of the arcuate fasciculus in the general population. The advent of diffusion tensor imaging (DTI) tractography that can visualize white matter pathways *in vivo* brought a major influx of information. It was shown that the anatomy of the arcuate fasciculus is more complex than previously thought (Catani, et al., 2005, 2007), and this will be discussed later on (Chapter 1.4). The following section will review the basics of DTI, and explain the limits of this technique, in order to subsequently introduce the contributions of DTI to the neuroanatomy of the arcuate fasciculus.

1.2 Diffusion Tensor Imaging Tractography

Our ability to study the perisylvian language pathways in the human brain is contingent upon the power of our methods of investigation. Using diffusion tensor imaging (DTI) we can garner indirect information on cell structure, and explore white matter connections of the living human brain (Jones, 2008). Hence, this section will focus on portraying the methodological advantages and limitations of diffusion tensor imaging and the related fibre tracking method, or tractography, used in this PhD study.

1.2.1 Introduction to white matter tractography

The study of human brain connections has a long history dating back from the 19th century's pioneering blunt dissections to involve staining techniques of degenerating myelin, axonal tracing, neurohistology and more recent cortical electrophysiology and DTI tractography.

Among the myriad of different MRI methods DTI offers the advantage of allowing the exploration of the brain's connectional anatomy *in vivo*. It is a non-invasive technique that measures molecular diffusivity inside tissues in order to probe tissue structure at a microscopic level, well beyond the usual image resolution of other *in vivo* imaging methods (Basser, et al., 1994; Pierpaoli and Basser, 1996; Le Bihan, 1985). Molecular diffusivity or *diffusion* is a random motion of molecules and represents an essential phenomenon in all living cells. DTI is based on the notion that the displacement of water molecules during diffusion is proportional to the "Apparent" Diffusion Coefficient (ADC) and the time. ADC thus represents an index of mobility of water molecules inside biological tissue. A visualisation of the diffusion tensor is a "diffusion ellipsoid", which represents the 3D probability of the displacement of water molecules. The profile of the diffusion ellipsoid is defined by the square root of the three eigenvalues. In DTI the diffusion is characterised by the magnitude (trace), directional variance (anisotropy), and the orientation (eigenvectors) of the anisotropic diffusion (Alexander, 2011). The most common diffusion measures that can be derived from diffusion information are the mean diffusivity (MD), indicating the average mobility of water molecules in a tissue, and fractional anisotropy (FA), reflecting the directionality of water diffusion, or in other words degree of anisotropy in water diffusion (Basser and Pierpaoli, 1996). These measures can highlight subtle anomalies in the organisation of white matter tracts that are not visible with anatomical MRI. One of the major advantage of DTI is that it is rotationally invariant (of the orientation of the subject/scanner), and that 3D alignments can be extracted easily and visualised using the 2D colour-coded scheme proposed by Pajevic and Pierpaoli (1999).

Diffusion MRI fibre tractography also referred to as fibre tracking or tract tracing, can be defined as the virtual reconstruction of the white matter pathways from diffusion MRI data. It is the most advanced visualisation strategy, and uses directional information from diffusion measurements to estimate the white matter trajectories. Tractography algorithms can be split into two major classes, deterministic and probabilistic tractography. Deterministic tractography is the focus of this section, and will be discussed in more detail.

The general principle of deterministic tractography algorithms is to use the directional information described by the diffusion tensor. A primary assumption is that the direction of greatest diffusivity (the major eigenvector) is roughly parallel to the local white matter fibre bundle direction (Alexander, 2011). If we assume that the main eigenvector is tangential to the underlying trajectory of the white matter, starting from a seed voxel we can propagate a 3D curve that represents the white matter pathway according to the tractography algorithm. This is called streamline tractography, originally proposed by Basser, et al. (2000) for diffusion tensor imaging. Several approaches have been presented for integrating the streamline pathway. The simplest are Fibre Assignment by Continuous Tracking (FACT) (Mori, et al., 1999) and Interpolated Streamline (IS) approach (Euler method, Runge-Kutta method, etc) (Basser, et al., 2000). Using FACT the tract follows a path parallel to the principal eigenvector until the end of the voxel. In FACT the step size is not fixed, and thus works well in low curvature regions. On the other hand, using IS at each step a new direction is interpolated taking into account the surrounding eigenvectors. With the IS approach the step size is fixed and is smaller than the voxel dimensions. Sub-voxel interpolation is necessary because we need a new direction in each new position. Criteria must be defined to terminate tracts when they either leave the tissue regions of interest or become unreliable. There are two main criteria on stopping the streamlines. The first one is the level of anisotropy, in order to avoid regions of low FA (e.g. less than 0.2) that can result in high noise effect and variability, and regions such as CSF and grey matter. The obvious limitation is that FA can be quite low in some white matter regions (e.g. due to crossing fibres), which can cause some tracts to terminate prematurely (Alexander, 2011). The second commonly used criteria is the angle of curvature - requiring that fibres do not turn too sharply, but follow anatomically reliable trajectories. Once we obtain our streamlines, we can use them as regions of interest to sample quantitative measures. At each step we can sample the value of FA, MD, etc. along the tract and perform quantitative analysis. Generally, tractography can be visualised in two different ways, as streamlines (Conturo, et al., 1999; Mori, et al., 1999; Basser, et al., 2000) or as streamtubes, i.e., cylindrical 3D tubes constructed by sweeping a circle along the corresponding streamline (Catani, et al., 2002, 2005). A streamtube approach is computationally more expensive in terms of display rendering than visualising streamlines (Leemans, 2011). However, it enhances the visual cue of the tract shape, and hence will be used in the figures presented in this PhD thesis.

1.2.2 Estimates of white matter changes: what does it all mean?

White matter is the translucent substance of the brain containing long and short range fibres connecting distant and local cerebral regions respectively. It is composed of mainly two population of cells: axonal propagations of neurons contained in the grey matter and non-neuronal cells. Myelin sheaths surround the axons and are composed of multiple segments of myelin, which are modified extensions of oligodendroglial cell processes (Barkovich, 2000). It is known that myelin can influence conduction velocity by regulating axon diameter, thickness of the myelin sheath, and the number and spacing of nodes of Ranvier. Hence, myelin can affect information processing in the brain by regulating velocity and synchrony of impulse conduction. Importantly, myelination continues for decades in the human brain and is modifiable by experience (Bengtsson, et al., 2008; Fields, 2008). Changes of the single constituents of the white matter substance, e.g. maturation of myelin, decreasing axonal water secondary to microtubule and microfilament production, or decreasing extracellular free water secondary to myelin production, happen as part of normal brain development. Pathological white matter changes have been described in many psychiatric conditions including depression and schizophrenia (Fields, 2008), visualised and quantified in vivo using diffusion MRI and diffusion measures such as FA, MD, perpendicular (radial) diffusivity, parallel (axial) diffusivity etc. Changes in the MRI signal are thought to reflect a number of physiological processes such as demyelination, oedema, gliosis, inflamation (Assaf and Pasternak, 2008). Nevertheless, there is still limited understanding of the link between the biology of white matter and the diffusion signal, with diffusion outcome measures lacking in specificity. Nevertheless, this section will try to highlight current agreements on the possible interpretations behind diffusion estimates of white matter changes.

It is important to distinguish the contributions of both the intra and extra-cellular compartments to measured diffusion indices. This is because at least two distinct populations of water molecules are known to contribute to MR images of white matter (Barkovich, 2000). The first is composed of water located within the myelin sheath, while the second is composed of intra-axonal and interstitial water (i.e. water outside of the myelin sheath). Myelin water can diffuse across axonal and myelin membranes and interact with water molecules in the other compartments. Recent research has identified that axonal membranes play the primary role in diffusion anisotropy by hindering the water diffusion perpendicularly. However, it is still not clear whether increases in anisotropy are due to reduced diffusivity along the axes perpendicular to it (Bhagat and Beaulieu, 2004; Bonekamp et al., 2006; Eluvathingal et al., 2007; Giorgio et al., 2008; Snook et al., 2005) or due to greater diffusivity along the main diffusion axis (Ashtari et al., 2007) or a combination of the two (Giorgio, et al., 2010). Myelin sheaths that surround the axons further modulate the degree of anisotropy, although myelination itself is not a requirement for the presence of significant anisotropic diffusion (Beaulieu, 2002). Many other factors can also modulate the estimates of water diffusion in brain tissue. These include methodological factors (e.g. movement artifacts), biological explanations (e.g. brain development) or pathological (e.g. white matter degeneration, demyelination, inflammation, etc.). A synopsis of recent research on possible interpretation of diffusion changes is presented in Table 1.2.2 with an emphasis on biological explanations. We have to bear in mind the limitations of interpreting these diffusion data, together with the fact that all these diffusion indices are intrinsically related to each other.

diffusion index	↓ DECREASE	↑ INCREASE
fractional anisotropy	• higher complexity of fibre organization (e.g. branching, crossing, kissing) • reduced fiber density • demyelination • reduced cohesiveness • reduced axonal membrane integrity	• progressive fibers organization • 'true' fibers myelination • increased axonal fibre diameter
mean diffusivity	• membrane proliferation (i.e. pre-myelination) • 'true' fibers myelination	• compromised axonal membrane integrity
perpendicular diffusivity	• thicker myelin (i.e. premyelination, overmyelination) • increased myelination - faster neural transmission • more water impermeant myelin • denser packing of fibers • smaller fiber diameters • progressive fibers organization	• delayed myelination (under-myelination) • loss of myelin (demyelination) • more water permeable myelin (dysmyelination) • loss of axonal membrane integrity (axonal damage) • more sparse packing of fibers (e.g. axonal dropout) • less coherent fiber packing (e.g. cellular remnants or incomplete pruning) • large-diameter fibers
parallel diffusivity	• membrane proliferation (i.e. pre-myelination)	• axonal loss • less dense fiber packing • progressive fibers organization

Table 1.2.2 Some of the likely biological interpretations of increases and decreases of diffusion indices. The list is not complete and is not intended as a comprehensive overview, rather as a guide; the different diffusion measures are not independent and are intrinsically related to each other.

Despite the importance of obtaining reliable tractography results, only a few studies have provided information regarding the reliability of tractography measurements (Ciccarelli et al., 2003; Danielian et al., 2010; Heiervang et al., 2006; Vollmar et al., 2010), with only one using deterministic tractography (Danielian et al., 2010). In general, test-retest reliability of other neuroimaging measures, such as the blood oxygenation level dependent (BOLD) signal in fMRI, will typically not be greater than 0.7 (Vul, et al., 2009). Further, the intersession reliability of diffusion measures depends on the structure studied (e.g. arcuate fasciculus versus corpus callosum) and the variable used (e.g. FA versus tract volume) (Wang, et al., 2012). In the above mentioned studies, the arcuate fasciculus, together with the corpus callosum, cingulum, cerebral peduncular fibres and uncinate fasciculus, are the structures that exhibit the most reliable diffusion measures (i.e. intersession coefficient of variation ≤ 10% or intraclass correlation coefficient ≥ 0.70), compared to fornix, inferior longitudinal fasciculus, inferior fronto-occipital fasciculus and so on. The findings from Danielian et al. (2010) and Wang, et al. (2012) further indicate that the number of gradient directions and scan repetition are both more important than the choice of MR scanner for test-retest reliability of tractography measurements, as well as for MRI signal variation and physiological noise/change over time.

1.2.3 Advantages and limitations of tractography data

Whilst tractography has many advantages over older techniques of studying brain connections a number of limitations still exist. Some of the advantages and limitations of tractography, in relation to other methods for studying connections (post-mortem), are summarised in Table 1.2.3.

method	↑ ADVANTAGES	↓ LIMITATIONS
tractography	• in vivo • advanced visualization method • applicable to human and animal brains • noninvasive • time efficient • allows the study of large populations • correlation with behavioural and other functional measures • quantitative • allows multiple hypothesis testing	• indirect anatomical method • low spatial resolution • presence of artifacts • operator dependent • limited visualization of bending, merging and crossing fibres • specificity of the outcome measures - limited understanding of the link between biology and signal • long acquisition times for the clinical application • gradient power • processing complexity - not allowing easy access to real time image and raw data for clinical application • inability to follow final cortical destinations of the tract

Table 1.2.3 Advantages and limitations of diffusion tractography method

There are many sources of errors that can confound tractography results. These include very small perturbations in the image data, i.e. acquisition noise, physiological noise, image distortions, scanner stability, head motion, partial-volume averaging, etc. (Alexander, 2011); inaccuracy of the single-tensor model to solve the regions of crossing, merging and bending of white matter fibres; dependency on a number of factors under the control of the experimenter, such as the choice of angular and anisotropy thresholds, and tractography algorithm (Catani and Dell'Acqua, 2011), and lastly presence of pathological processes, such as brain oedema, bleeding, and compression which could all affect tractography results. Improving diffusion acquisition (higher spatial resolution and signal to noise ratio), processing (complex fibre orientation, tractography algorithms) and specificity of the outcome measures in relation to the underlying biology, could lead to better application of this technique in research and clinical settings. Nevertheless, and, despite its' limitations, deterministic tractography has allowed anatomical models of language to be re-explored, and permitted the visualisation and assessment of the microstructural integrity of the perisylvian

language pathways in the living human brain that conventional structural and functional MR imaging were not able to provide. In this PhD thesis two applications of tractography will be used: first, to explore the perisylvian language pathways in a healthy population, and second to investigate the perisylvian language pathways relative to brain pathology. The contributions of DTI tractography to the anatomy of the perisylvian language pathways will be discussed in the following section.

1.3 Arcuate fasciculus: anatomy and function

The neural basis of language has been the topic of research for over two hundred years (see Section 1.1 of this Chapter). Since then our ideas about how language is processed in the human brain have undergone many changes mostly due to the technological advances that are driving research possibilities. The historical cortico-centric view, which included only Broca's and Wernicke's area in a language model, has been supplemented by models that assign language-related functions also to white matter pathways such as the arcuate fasciculus, and subcortical structures (e.g. basal ganglia, thalamus). Consequently, the anatomy and function of the arcuate fasciculus became of crucial importance in the neuroscience of language, and these will be discussed in a more detail below.

1.3.1 Arcuate fasciculus: contributions of diffusion tensor imaging tractography

The arcuate fasciculus is regarded as a white matter tract that lies in the inferior portion of the superior longitudinal fasciculus (SLF), superior to the insula and extreme capsule, connecting temporal and frontal language areas (Shuren, 1995). Recently, diffusion tensor imaging (DTI) tractography has permitted the visualisation and assessment of this large white matter pathway in the living human brain that conventional structural and functional MR imaging were not able to provide (Catani, 2007, 2005; Kamada, 2007; Makris, 2005; Mamata, 2002; Nucifora, 2005; Parker, 2005; Powell, 2006; Rivkin, 2000; Vernooij, 2007). However, because of the proximity of the arcuate fasciculus and the SLF III (the perisylvian component of the SLF connecting supramarginal gyrus with the ventral premotor and prefrontal regions (Thiebaut de Schotten, et al., 2011a)), and the limitations of the current DTI methodology, these two pathways are indistinguishable in tractography dissections (Frey, et al., 2008; Friederici, 2009). Hence, the two terms - arcuate fasciculus and SLF might be used interchangeably in this PhD thesis.

DTI tractography has shown that the anatomy of the arcuate fasciculus is more complex than previously thought (Catani, et al., 2005, 2007; Martino, et al., 2012). Besides directly connecting classical language areas in the frontal and temporal lobes, studies have shown that inferior parietal areas might also be part of the arcuate fasciculus' network. Hence, the proposed connection pattern of the arcuate fasciculus encompasses the network of fronto-temporo-parietal fibres, which is the neuroanatomical approach followed

in this PhD thesis. In other words, this parallel pathway model describes two distinct parallel pathways that interconnect cortical language territories of the frontal, temporal and parietal lobes (Catani, et al., 2005). The classical direct pathway (i.e. the arcuate fasciculus *sensu strictu*), or the *direct long segment* (denoted in red in Fig 1.3.1), connects the inferior frontal cortex (Broca's territory) with the superior temporal cortex (Wernicke's territory). The indirect pathway is composed of two segments: the *indirect anterior segment* (denoted in green in Fig 1.3.1), which connects Broca's territory with the inferior parietal lobule (Geschwind's territory), and the *indirect posterior segment* (denoted in yellow in Fig 1.3.1) that connects Wernicke's territory with Geschwind's territory (Catani, et al., 2005). The reason why these regions were named 'territories' and not the classical 'areas' lies in the fact that tractography revealed unexpected projections of the arcuate fasciculus, whose cortical terminations extended beyond the classical limits of Wernicke's (BA22, 37, 39, and 40) and Broca's (BA 44 and BA45) areas to include part of the posterior middle temporal gyrus, middle frontal gyrus and inferior precentral frontal gyrus, respectively (Catani, et al., 2005). Geschwind's territory, now important as a separate primary language area, corresponds to BA39 and 40, and although its importance as a language region has been recognised for some time (Geschwind, 1965), the exact role of this area is still largely unknown (Catani, 2009) (more on the functions in the following Section 1.3.2). It is important to note that the support for the existence of the indirect pathway, with connections to the Geschwind's territory, came from the classical post-mortem dissections (Lawes, et al., 2008), human intraoperative electrocorticography (Matsumoto, et al., 2004), functional MRI studies (Bullmore, et al., 2000; Schmithorst and Holland, 2007), studies of the homologous parts of the monkey brain (Deacon, 1992), and lastly DTI studies (Lawes, et al., 2008; Eluvathingal, et al., 2007; Maldonado, et al., 2012).

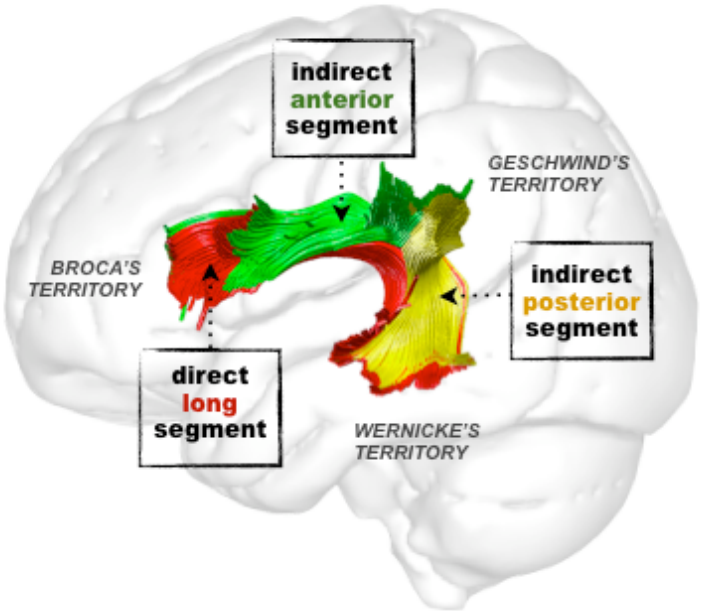

Fig 1.3.1 Tractography dissections of the three segments of the left arcuate fasciculus: direct long segment (in red), indirect anterior (in green) and indirect posterior segment (in yellow).

In the last two decades the boundaries of the language network were expanded beyond the arcuate fasciculus. One of the most interesting development has been the demonstration that areas in the medial, inferior and anterior temporal cortices, traditionally considered outside the classical language network, may play a crucial role in semantic processing. The interaction of these additional areas with the canonical perisylvian language network may be mediated by a set of ventral tracts such as the inferior longitudinal fasciculus, the uncinate fasciculus, and the inferior fronto-occipital fasciculus (Catani and Mesulam 2008). Although this PhD thesis investigates only the arcuate fasciculus, these additional connections that play a role in language will be described briefly. The inferior longitudinal fasciculus connects the anterior temporal pole and the occipital lobe, and is likely to be involved in visual object recognition, semantic processing and linking of object representations to their lexical labels (Catani, et al., 2003; Mandonnet, et al., 2007; Mummery, et al., 1999). The uncinate fasciculus, connects the anterior temporal lobe with the orbitofrontal area, including inferior frontal gyri. It may play a role in lexical retrieval, semantic associations, and aspects of naming that require connections from temporal to frontal components of the language network (e.g. the naming of actions) (Catani and Mesulam, 2008). The inferior fronto-occipital fasciculus is part of the mirror neuron system and arguably the only direct connection between frontal and occipital cortex in the human brain (Forkel, et al., in press). The functions of this pathway are not fully understood, but may involve reading and writing and other semantic aspects of language (Duffau, 2012; Duffau, et al., 2005). The extreme capsule tract is a ventral pathway that has been initially described in monkey and more recently in humans with diffusion tractography (Parker et al., 2005; Saur et al., 2008). However, the term extreme capsule tract is not specific enough as many other tracts run through the extreme capsule, including the uncinate fasciculus, the inferior fronto-occipital fasciculus and many fibres connecting to the insula. Future studies are necessary to establish the presence of direct connections linking Wernicke's to Broca's territory running through the extreme capsule. Besides mentioned ventral connections, the frontal aslant tract is a newly described pathway connecting posterior Broca's region with medial frontal areas including pre-supplementary motor area and cingulate cortex. This tract is left lateralised in most right-handed subjects, suggesting a role in language (Catani et al., 2012). In addition to the above tracts, data from axonal tracing studies and tractography have suggested the existence of additional tract important for language, the middle longitudinal fasciculus. This tract is an important association tract between posterior temporo-parietal areas and anterior temporal regions, and thus it is likely involved in linking sounds to meaning (Makris, et al., 2009; Menjot de Champfleur, et al., 2012). Nevertheless, unilateral resection of the middle longitudinal fasciculus does not result in permanent language deficits (De Witt Hamer, et al., 2011). Besides these white matter pathways, there are other structures that play an important role in language processing, such as: posterior third of the corpus callosum - for interfacing syntax and prosody (Friederici, et al., 2007), and subcortical structures such a basal ganglia (caudate nucleus) - for language comprehension that requires less automatic and more controlled language processes (Crinion, et al., 2006; Friderici, 2006a; Ullman, 2004) and thalamus - for processing syntactic and semantic language analysis (Wahl, et al., 2008). Having touched upon some functional correlates of the above mentioned brain structures, I will now discuss in more detail the possible functional correlates of the arcuate fasciculus.

1.3.2 Arcuate Fasciculus: possible functional correlates

Mapping functions onto a single tract is subject to the same criticism directed at localisationism. Our knowledge of possible functional correlates of the arcuate fasciculus depends upon the specific temporal and/or spatial context (stage of development, injury, disorder, method of inquiry, etc.), and the neural systems affected. Nevertheless, diffusion tractography combined with functional MRI is likely to offer productive insights into the structure-function relationship of this perisylvian language network.

Knowing that the arcuate fasciculus connects the inferior frontal, temporal and inferior parietal cortices, the functions assigned to the above mentioned cortical regions will now be briefly discussed, as they provide the first clue to the possible functional correlates of the white matter pathway in question. Before going into each specific cortical region in more detail, it is important to note that there are hemispheric differences in processing linguistic information (more on language lateralisation in Section 1.4 of this Chapter). Complementary evidence from neuropsychological and neuroimaging studies suggest that language is processed via an effective basic bilateral system, which is superimposed on the left-dominant perisylvian language system (Bozic, et al., 2010; Brauer, et al., 2008). Bozic, et al. (2010) proposed that speech comprehension is supported by bihemispheric fronto-temporal system - supporting sound-to-meaning mapping and general perceptual processing demands, and a more specialized left hemispheric perisylvian system supporting morpho-syntactic functions. However, research showed that the left hemispheric perisylvian network might also be responsible for processing lexico-semantic and not just morpho-syntactic information (Friederici, 2002, 2006b). In contrast, the right hemisphere is thought to be responsible for processing prosodic information (Meyer, et al., 2002; Zatorre, et al., 2002). Linguistic prosody is mainly localised to the right hemisphere, unless phonemic segmental information is present in a speech signal, and prosody is segmentally bound (Friederici, et al., 2007) - then the left hemisphere comes into play. I will briefly summarise regional differences in processing linguistic information, starting from the classical language areas, Broca's and Wernicke's, to the inferior parietal lobule, before proceeding to the possible functions of direct and indirect pathways of the arcuate fasciculus.

Broca's area is located in the left inferior frontal cortex, and comprises the opercular (BA44) and triangular (BA45) parts of the inferior frontal gyrus (Amunts, et al., 2004). Neighbouring areas include premotor area 6 at the ventral precentral gyrus, dorso-lateral prefrontal areas 9 and 46, area 47, and the anterior insula (Amunts, et al., 2010). Recently, Broca's area was successfully parcellated using transmitter receptor distribution (Amunts, et al., 2010), high angular resolution diffusion imaging (Frey, et al., 2008) and probabilistic tractography (Anwander, et al., 2007) to reveal anatomical dissociation between BA44 and BA45, which proved to be functionally relevant. In the past, Broca's area has been considered as the brain centre responsible for speech production. However, with advances in neuroimaging, combined with the neuropsychological evidence, it has become clear that Broca's area is involved in a wider range of functions. These include syntactical and phonological analysis, verbal fluency, semantic retrieval and categorization, language comprehension, selection between competing linguistic alternatives, mathematical calculation,

music processing and understanding actions of other individuals (Amunts, et al., 2004; Bozic, et al., 2010; Fazio, et al., 2009; Friederici, 2011; Sakai, 2005). It is thought that in healthy adults the anterior ventral regions (BA47/45) support processing of lexical semantics, whereas posterior dorsal regions (BA45/44) support syntactic processing (Bookheimer, 2002; Bozic, et al., 2010; Friederici, 2002). Wernicke's area, encompassing the middle and superior temporal gyri, has been considered to play a role in language comprehension for over a century. It is known that temporal activation of Wernicke's area appears earlier than Broca's during language comprehension, but vice-versa during language production (Brauer, et al., 2008). This bidirectionality, subserved by the arcuate fasciculus, has also been shown in a recent human intraoperative electrocorticography study (Matsumoto, et al., 2004). However, the functions of Wernicke's area are also much wider than previously thought. More specifically, the posterior middle temporal region is associated with storing lexical-semantical representations, anterior superior temporal cortex with building of syntactic structure, and posterior superior temporal cortex for semantic-syntactic integration during sentence processing (Friederici, et al., 2011). Recent imaging studies have shown that inferior parietal lobe (Geschwind's territory) is another crucial part of the perisylvian language network. It is thought to play a role in control of intention to speak (Carota, et al., 2010; Desmurget et al., 2009), speech self-awareness (Jardri, et al., 2007), production of gestures related to tools and speech planning (Damasio and Damasio, 1992; Daprati and Sirigu, 2006; Haaland, et al., 2000), word semantics and conceptual semantics (Friederici, et al., 2011), verbal working memory (Jacquemot and Scott, 2006), and global coherence of narratives (Martin-Loeches, et al., 2008). Thanks to its anatomical position, Geschwind's territory is considered to be a convergence and integration zone for sensory and motor information and their temporal dynamics (Catani, 2009). However, although research has provided us with valuable insights, the exact functional roles of these language cortical regions remain elusive. Even less is known about the functional correlates of the perisylvian language pathways.

Lesion studies in patients with perisylvian damage indicate that the arcuate fasciculus is involved in almost all aspects of language and verbal working memory. In the light of the parallel pathway model, and the combined evidence from aphasic patients, Catani et al. (2005) suggested the following functions for direct and indirect pathways of the arcuate fasciculus: the direct pathway subserves phonologically based language functions (e.g. automatic repetition), while the indirect pathway facilitates semantically based language functions (e.g. vocalisation of semantic content, auditory comprehension, etc.). The conclusions were mainly derived from observing impaired repetition but relatively preserved spontaneous speech and language comprehension in conduction aphasia (lesions in parts of the direct pathway); but intact repetition and impaired spontaneous speech and language comprehension in transcortical motor aphasia (lesions in the indirect pathway). However, recent findings question the supremacy of the long segment in word repetition, and show that the contributions of the direct and indirect pathways in repetition remain to be clarified (Berthier, et al., 2012; Breier et al., 2008; Epstein-Peterson, et al., 2012; Friedrikssen et al., 2011). In this two-route model of language processing, Catani et al. (2005) suggested that lesions of different indirect pathways would produce different symptoms. Hence, a lesion of the indirect anterior segment would result in a failure to vocalise semantic content, while a lesion of the posterior segment would result in a failure of auditory semantic comprehension. Recent findings give support to this model, and show that lesions

involving Broca's area and anterior segment lead to Broca-like conduction aphasia, while lesions to the Wernicke's area and posterior segment produce Wernicke-like conduction aphasia (Song, et al., 2011) However, very often aphasia patients present with extensive lesions, which makes it difficult to attribute specific functions to single segments of the arcuate fasciculus. The combined analysis of structural and functional connectivity of perisylvian networks in healthy subjects may represent a valid alternative. In a recent study Lopez-Barroso et al. (2012) showed that performance in word learning correlates with microstructural properties and strength of functional connectivity of the direct connections between Broca's and Wernicke's areas. There were no correlations with the segments of the indirect pathway. This study demonstrates that our ability to learn new words relies on efficient and fast communication between temporal and frontal areas. Schulze et al. (2012) suggested that the absence of these connections in other animals may explain human unique ability to learn words and connect them into meaningful sentences. While the direct pathway may support auditory-motor integration, which is crucial during early stages of language acquisition, the role of the indirect pathway may be more complex and more relevant during later stages of language development, such as linking semantics and phonology (posterior segment) (Newhart, et al., 2012; Parker, et al., 2005), processing syntactically complex sentences (Newhart, et al., 2012; Perani, et al., 2011; Wilson, et al., 2011) and various aspects of verbal working memory (Burzynska, et al., 2011; Vestergaard, et al., 2011). There is further evidence of the arcuate fasciculus being involved in the development of reading skills (Rimrodt, et al., 2010; Yeatman, et al., 2011), however how specific segments play a role in reading remains elusive.

The parallel pathway model brings together the opposing views on the functional correlates of the arcuate fasciculus, with some suggesting it supports the processing of complex syntax (Brauer et al, 2011; Friederici, 2009; Friederici, et al., 2006a, 2011; Griffiths, et al., 2012), and others that it supports language repetition by auditory-motor mapping (Saur, et al, 2008, 2010; Hickok and Poeppel, 2007). Nevertheless, additional diffusion and functional MR imaging studies are needed to further clarify the role that each segment of the arcuate fasciculus has in language processing. This PhD study provides an important step forward in our understanding of the role that specific segments of the perisylvian network have in language dysfunction in autism spectrum disorders.

1.4 Introduction to language asymmetry

Asymmetry of the arcuate fasciculus is one of its key features. This section gives a general introduction to the concept of language lateralisation, in order to provide a background for the first tractography study (see Chapter 2), which explores the maturation effects on the asymmetry patterns of the perisylvian language network.

Hemispheric lateralisation of the human brain has, for a long time been the focus of interest in numerous fields of neuroscience. From an evolutionary prospective, Vallortigara (2006) regards the brain lateralisation at the population level as an "evolutionary stable strategy" that might increase brain efficiency. Denenberg (1981) is in agreement that the advantage of lateralisation is to increase neural capacity. Specialising one hemisphere for a particular function leaves the other hemisphere free to perform other additional functions. Thus, useless duplication of functions in the two hemispheres can be avoided and neural tissue spared. Language is one of the most known lateralised brain functions. Since 1865, when Broca coined the motto: "one speaks with the left hemisphere" (Broca, 1865), the left hemisphere has been regarded as the neural substrate for core language skills. In humans language lateralisation can be functional and structural.

Functional hemispheric lateralisation for language has been shown to correlate with handedness in several neuroimaging studies using functional MRI, positron emission tomography (PET) and magnetoencephalography (MEG), where approximately 95% of right-handers show left-sided functional lateralisation, while 15% of left-handers show right-sided functional hemispheric lateralisation (Vernooij, et al., 2007; Springer, et al., 1999; Pujol, et al., 1999). Minagawa-Kawai et al. (2008) used near-infrared spectroscopy to show that 85% of his right-handed subjects exhibit left dominance in response to auditory speech stimuli. During the first year of life there is, at rest, no reported left-right difference in cerebral blood flow in linguistic regions (i.e. inferior frontal, superior temporal and plurimodal temporal – parietal regions). However in response to auditory stimuli, asymmetric response favouring the left side is observed in fMRI and event-related potential studies (Dehaene-Lambertz, et al., 2002). Significant left asymmetry is present for speech-like stimuli from birth onwards, and it is suggested that during the first months of life the left auditory areas are more reactive than the right areas to any sound, and this bias can contribute to orientation towards the left hemispheric processing of speech. Locke et al. (1995) considers babbling as the process that marks the onset of the left hemispheric control of speech-like activity, and begins around 6-7 months together with the sharp increase in rhythmic hand movements and right-handed reaching. Left hemisphere advantage in processing speech can also be studied with dichotic listening, which is based on the competing message technique when two different verbal stimuli are presented one to each ear and selective attention is then assessed. With this method, right ear advantage was found in children after 3 years of age (Ingram, 1975; Kimura, 1967). Language asymmetry during development will be discussed in more detail in Chapter 2.1.3.

Although this functional lateralisation of language has been well documented, the neuroanatomical basis for it has not yet been fully elucidated. Forty years ago, Geschwind and Levitsky (1968) reported a greater left planum temporalis in approximately 65% of the normal population. Since then various studies found structural asymmetries in a number of brain regions relevant to language processing (Vernooij, et al., 2007). However, a direct link between structural and functional lateralisation has not been clearly established. The incidence of left-hemisphere planum temporale asymmetry (about 60-80%) is lower than the incidence of left hemisphere language lateralisation in the population as estimated by functional studies (›90%) (Dorsait-Pierre, et al., 2006). Other anatomical left asymmetries observed at the macroscopic and cytoarchitectonic levels include a longer left Sylvian fissure (Geschwind and Levitsky, 1968); larger left inferior frontal region, though less frequent (Knaus, et al., 2006); greater white matter volume underlying Heschl's gyri (Penhune, et al., 1996); bigger pyramidal cells in the left auditory cortex (Hutsler, 2003) and these are associated with thicker myelinated fibres (Anderson, et al., 1999), and lastly, widths of the individual cortical columns and distances between those columns are greater in the left superior temporal lobe (Seldon, 1981).

The lateralisation pattern of the arcuate fasciculus is somewhat more complex. DTI tractography revealed a highly heterogeneous distribution of the lateralisation pattern for the long segment of the arcuate fasciculus in the healthy human population (Catani, et al., 2007). An extreme degree of left lateralisation (as measured by the number of streamlines as an indirect index of volume) was found for the direct long segment in 60%, mild left lateralisation in 20% and symmetrical pattern in 20% of the normal adult right-handed male and female population (Catani, et al., 2007). The degree of lateralisation of the long segment in left-handed individuals is less clear, with some suggesting that it is similar to the right-handed subjects (Vernooij, et al., 2007), and others that it is more bilateral (Hagmann, et al., 2006). Left asymmetry was also noticed for diffusion measures of the arcuate fasciculus, such as FA and MD (Powell, et al., 2008; Rodrigo, et al., 2007). Many diffusion studies confirmed the dominant left asymmetry of the arcuate fasciculus (Nucifora, et al., 2005; Barrick, et al., 2007; Parker, et al., 2005; Powell, et al., 2006; Upadhyay, et al., 2008; Vernooij, 2007). The left asymmetry of the long direct segment is already present in children between 6-17 years of age (Eluvathingal, et al., 2008). Upadhyay et al. (2008) confirmed this left asymmetry in FA and fibre density of the long direct segment in healthy right-handed adults. Furthermore, the authors also used diffusion tensor spectroscopy and showed that radial diffusivities (RD) of NAA (N-acetyl-aspartate) and water were both lower in the left arcuate fasciculus, but only significantly lower for RD (NAA). Since intra-axonal properties primarily determine RD (NAA) it is possible that an intra axonal difference exists between the two fascicules. Upadhyay et al. (2008) suggest that larger axonal diameters of the left arcuate fasciculus may explain this RD (NAA) difference, which in turn suggests that the left arcuate fasciculus is responsible for a fast phasic signal.

An important question is whether this anatomical lateralisation of the long segment of the arcuate fasciculus is related to the functional lateralisation of language. Preliminary studies combining DTI tractography and fMRI show no correlation between the lateralisation of the long direct segment volume and the degree of functional lateralisation as determined by fMRI during tasks of verbal fluency, verb generation and reading comprehension (Powell, et al. 2006; Vernooij, et al. 2007). However, the lateralisation of the

fractional anisotropy values of the long segment seems to correlate better with the functional lateralisation as demonstrated in healthy individuals (Powell, et al. 2006) and in patients with temporal lobe epilepsy (Rodrigo, et al. 2008).

Significant differences in lateralisation pattern of the long direct segment are present between genders, with females more likely to have a symmetrical bilateral lateralisation pattern than males (Catani, et al., 2007). This symmetrical distribution of the long segment was correlated with better performances in complex verbal memory tasks. These findings are not surprising having in mind that gender differences were previously reported for the lateralisation of the volume of cortical language regions (Good et al., 2001; Luders et al., 2004), subcortical white matter anatomy (Good et al., 2001; Hagmann et al., 2006), and activation patterns during linguistic tasks (Shaywitz et al., 1995). Some studies suggested that different maturational trajectories are driving these gender differences in lateralisation (Paus, 2009; Perrin et al., 2009), but this was not confirmed in a recent tractography study of children and adolescence (Lebel and Beaulieu, 2009). Our first tractography study (see Chapter 2) will address some of these issues.

Other components of the perisylvian networks seem to have a more bilateral distribution (posterior segment) or right lateralisation (anterior segment). Inter-hemispheric differences have been found in the fractional anisotropy of the anterior indirect segment with higher values in the right side (Catani, et al. 2007; Eluvathingal, et al. 2007), and this was later confirmed also for the volume measures (Thiebaut de Schotten, et al. 2011b). This right lateralisation of the anterior segment may be related to the specialization of the right parietal and frontal cortex for visuo-spatial processing (Doricchi, et al., 2008; Thiebaut de Schotten, et al., 2008).

In conclusion, tractography studies suggest an overall prevalence of left asymmetry for the long direct segment of the arcuate fasciculus in approximately 80% of the population. Considering that the prevalence of left functional dominance for language is >90%, asymmetry of the long direct segment may represent a more critical anatomical substrate for language lateralisation than planum temporale asymmetry, whose leftward lateralisation is found only in around 65% of the right-handed population (Geschwind and Levitsky, 1968).

1.5 Language evolution: the rise of the arcuate fasciculus

Language is a defining feature of the human species, and yet little is known about how this ability developed. This PhD study adds indirect information to the phylogenic context of language anatomical development, through inferences from language-brain relationship during human ontogeny, and heritability of language related brain structures. Hence, the first tractography study on the maturation of the arcuate fasciculus could be informative for language evolution (see Chapter 2), as well as investigating the heritability of the perisylvian connections (see Chapter 4). Furthermore, systematic analysis within clinical syndromes associated with social and language disorders could provide new insights into language evolution. Hence, the study on autism and the perisylvian language network might bring new insights (see Chapter 6). Thus, this section will give a general blueprint of the recent developments in the evolutionary research of language, focusing on the arcuate fasciculus in order to find its place in a phylogenic frame of reference.

1.5.1 Paleoneurological evidence for the origins of language brain regions

There are several ways to approach the study of language evolution. Due to the impossibility of direct examination of the brains of our predecessors, science had to turn to indirect methods of investigation, which are far from optimal. One way to study ancestral systems for language is through paleoneurology, a scientific field that uses fossil records to approach the study of brain evolution. The paleoneurological evidence is exciting, but has inherent problems given the often incomplete, fragmented, and eroded cranial portions of our fossil ancestors (Holloway, 1983). For example, KNM-ER 1470 specimen found in Kenya (originally assigned to *Homo habilis*, and later to *Homo rudolfensis*, which has an estimated age of 1.9 million years) suggested a unique sulcal pattern in the left inferior frontal gyrus (Broca's area) as being similar to modern humans and unlike Australopithecus and living great apes (Falk, 1983). However, the fossil evidence was difficult to interpret because the markings on the brain casts were very faint. Hence, no clear evidence exists for the presence of Broca's area in a specimen as geologically old as *Homo rudolfensis*. Some believe that Broca's area was present in the *Homo erectus* (specimen WT 15000, also called Turkana Boy, 1.5 million years old) noted by a slight slant on the cranium (Walker and Shipman, 1996). Thus, it is believed that at 1.5 - 2.0 million years ago there is a clearer fossil evidence for a Homo lineage showing a more modern and enlarged third inferior frontal convolution and strong cerebral asymmetries identical to those known for modern *Homo sapiens* (Holloway, 1983). However, the question is whether skull morphology reflects brain features at all. A sceptic would now remember the notorious fall of the phrenologists in the 19th century. These findings remain mere speculation and rest on weak scientific grounds. Nevertheless, these exciting discoveries, if true, would generate a number of implications regarding the existence of Broca's area and possibly the arcuate fasciculus very early in human evolution. But even is strong evidence existed for an enlarged third inferior frontal convolution so early in human evolution, it would still not imply that language was formed and used at that time. Many believe that language coincided with the modern humans once culture and complex social groups were established (Crystal and Varley, 2006) and Cro-Magnon man (species *Homo Sapiens*) appeared in Europe some 120,000 years ago.

1.5.2 Language evolution in genetic terms

Comparative genomics has brought about novel technologies, in particular microarrays, which are able to detect expression levels for thousands of genes simultaneously. As a result, it was revealed that the genome of our closest living relative, the chimpanzee, is astonishingly similar to our own, with approximately 96% of genes identical to our own (Cheng et al., 2005). Hence, many of the fundamental differences between humans and chimpanzees are likely to depend more upon differences in the regulation of gene expression than on differences in the amino-acid sequences of gene products (King and Wilson, 1975). This notion is supported by recent research describing human brain evolution in terms of an accelerated rate of change, up-regulation of gene expression, and a preponderance of changes in the regulation of genes affecting synaptic transmission and energy metabolism (Oldham and Geschwind, 2006).

To explore the importance that changes in the regulation of gene expression have had in the human evolution of language, microarray studies were used to compare expression levels for thousands of genes in the language brain areas between humans and chimpanzees. By applying this approach to the perisylvian network of brain regions involved in language, it may be possible to discern the genetic basis of language evolution in the brain. The research showed that grey matter of the left prefrontal cortex had significantly higher rate of change in gene-expression across species (humans and chimpanzees) compared with liver (Enard, et al., 2002; Gu and Gu, 2003). Furthermore, the large majority of genes are differentially expressed across species in various frontal and temporal cortical brain regions including language areas, with genes up-regulated in humans (Caceres, et al., 2003). However, a recent study by Khaitovich et al. (2004) found only a small number of transcripts with expression patterns unique to Broca's area in humans versus chimpanzees. These surprising results may however reflect methodological limitations, and the small number of individuals used in the study. Geschwind (2000) suggested that it is also possible that differences in cellular density and/or composition may underlie the functional and structural specialization of language areas in humans and that these differences fall below the detection limits of the microarrays, hence making this method unsuitable for exploration of human language evolution in genetic terms.

1.5.3 Comparative anatomy of the arcuate fasciculus

In order to understand how and why language evolved in the human species, and being unable to observe language evolution directly, we have to rely on comparisons between human and non-human primates. By comparing human and simian connectional anatomy of the language pathways we may unveil the evolutionary changes underlying the development of language. The comparison between human and non-human DTI data with respect to the arcuate fasciculus has raised questions of the extent to which differences and similarities are functionally relevant for language processing in humans (Friederici, 2009; Ghazanfar, 2008). This section explores recent research on comparative anatomy of the arcuate fasciculus.

Early 20th century comparative anatomy studies emphasised the essential role that the frontal lobes had for human cognition, justified by the great expansion of the frontal lobes along the phylogeny scale that seemed bigger than for any other brain region (Thiebaut de Schotten, et al., 2012). The large size of the frontal lobes was linked to extraordinary cognitive development, including development of language in humans. It was not known whether these anatomical differences were global or specific to certain frontal lobe pathways like the arcuate fasciculus. However, recent research challenges this supremacy of the frontal lobes (Théodoridou and Triarhou, 2012). It was revealed that the frontal cortex of humans and great apes occupies a similar proportion of the cortex (Semendeferi, et al., 2002), and moreover that the enlargement of the human brain has preserved the relationship between its major lobes compared to other species (Risberg, 2006). These similarities between human and non-human primates seem to stretch further to include an asymmetry in the planum temporale, Broca's area and lateral sulcus (Cantalupo and Hopkins 2001; Cantalupo et al. 2003; Galaburda et al. 1978; Gannon et al. 1998; Hopkins, et al., 1998). Although in terms of gross morphology human and simian brain language regions seem alike, anatomical differences were recently revealed at the microanatomical and molecular level. Buxhoeveden et al. (2001) discovered that increased width and spacing of cortical minicolumns in the left planum temporale of humans was absent in chimpanzees. These observed differences and similarities across species led to two opposing views on comparative anatomy of the language brain regions. One view holds that the neuroanatomy of the human and non-human brain is the same, and hence that fibre tracts of the monkey's brain are directly linked to the human language pathways. For example, Schmahmann et al. (2007) questioned whether arcuate fasciculus has a role in language at all because their monkey data showed different cortical terminations of the arcuate fasciculus, which did not connect mid superior temporal region to the Broca's homologue; rather, they proposed a pathway through the extreme capsule to be crucial for language. The other view holds that there are significant neuroanatomical differences across species that are crucial for language development in humans. This view acknowledges that there are similar brain phenotypes between humans and chimpanzees as a result of precursors for language structures that exist in chimpanzees, and probably existed in the last common ancestor of humans and chimpanzees (Oldham and Geschwind, 2006). Rilling et al. (2008) compared humans, chimpanzees and macaques, and analysed three pathways; the arcuate fasciculus and the superior longitudinal fasciculus as the dorsal connection, and the extreme capsule as the ventral connection. They found the arcuate fasciculus to be smaller in chimpanzees and absent in macaques. This dorsal pathway in humans terminated in the temporal lobe, whereas in chimpanzees, terminations were dominant in the parietal lobe. As a result, the authors argued that the expanded dorsal pathway in humans is crucial for the evolution of language. This finding supports the theory that changes in the strength of perisylvian connections between posterior temporal and inferior frontal regions have increased during evolution from monkey to human (Aboitiz and Garcia, 1997a). The theory rests on two evolutionary tendencies: the posterior temporal and inferior parietal regions became increasingly connected, linking the auditory system and a pre-existing parietal – premotor system; and development of connections between superior temporal and inferior frontal regions that link auditory information to orofacial premotor regions. There are speculations that these tendencies correspond to the evolution of posterior and long segments of the arcuate fasciculus, respectively, and the anterior segment being, phylogenetically, the oldest component of the perisylvian network (Catani, 2009).

Perisylvian connections in the monkey brain have been studied extensively using axonal tracing techniques; however, their significance with respect to language remains controversial because the homologies between cortical areas in monkeys and humans are unclear (Schmahmann and Pandya, 2006). Old axonal and recent tractography studies support the theory that evolution of language from monkey to human involved a change in a pre-existing pattern of perisylvian connections (Catani, 2009). Thiebaut de Schotten, et al. (2012) used spherical deconvolution tractography (Tournier et al., 2004; Dell'Acqua et al., 2007) to build an atlas of human frontal connections for a direct comparison with a recent atlas of the fibre pathways of the monkey brain (Schmahmann and Pandya, 2006). The authors investigated the third branch of the superior longitudinal fasciculus (SLF III) that connects the intraparietal sulcus and inferior parietal lobule to the inferior frontal gyrus (BA 44, 45, 47) and is equivalent to the anterior segment of the arcuate fasciculus. In the monkey the SLF III had a similar anatomy, linking the posterior part of the inferior frontal gyrus (area 6V and area 44) to the rostral portion of the inferior parietal lobule. The authors concluded that overall, the anatomy of the anterior segment of the arcuate fasciculus is highly conserved between humans and monkeys, in line with the theory that the anterior segment is phylogenetically the oldest component of the perisylvian network. The same study explored the comparative anatomy of the long segment of the arcuate fasciculus connecting posterior regions of the frontal lobe to the temporal lobe (Catani et al., 2002, 2005). In the human brain, a subset of connections links the most posterior part of the superior temporal gyrus (BA 41 and 42) to the inferior frontal gyrus (BA 44 and 45), while a larger subset of connections links the middle and inferior temporal gyri (BA 21, 22 and 37) to the inferior pre-central (BA 6) and posterior regions of the middle and inferior frontal gyrus (BA 8, 9, 44 and 45) (Thiebaut de Schotten, et al., 2012). However, in the monkey brain, the arcuate seems to connect only the caudal part of the superior temporal gyrus and the dorsal part of area 8, area 46, and area 6. In contrast, Yeterian et al. (2012) made a claim for the existence of a direct projection between the superior temporal lobe and dorsal prefrontal areas via the arcuate fasciculus in the monkey brain. Nevertheless, the described pathway is more likely to be a connection linking temporal areas to the eyes in order to orient the eyes to the sound. In conclusion, it seems that the classical long direct segment of the arcuate fasciculus is what makes human and monkey brain different (in terms of 'language' connections). Thiebaut de Schotten et al. (2012) confirmed that there are significant differences in this fronto-temporal segment of the arcuate fasciculus between human and monkey brains, with the projection to middle and inferior temporal gyrus being absent in the monkey (see Fig 1.5.3).

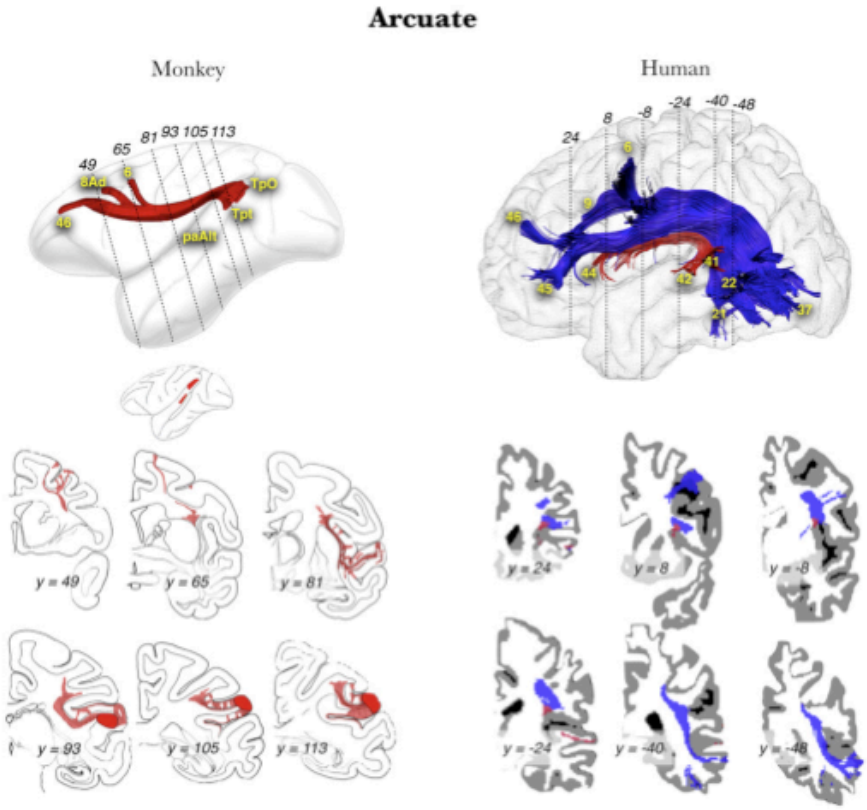

Fig 1.5.3 (adapted from Thiebaut de Schotten, et al., 2012) Reconstruction of the arcuate fasciculus: comparison between post-mortem axonal tracing in monkey and human in vivo spherical deconvolution tractography. Common anatomical features between human and monkey are reconstructed in red whereas anatomical differences have been coloured in blue.

Controversy surrounding language evolution involves two existing views divided according to whether the emphasis is placed on a hand-gestural communication ancestral system, or auditory-vocal ancestral mechanisms. The arcuate fasciculus is likely an element involved in auditory–vocal coordination and articulatory control, which did not arise out of nothing. According to Aboitiz (2012) these perisylvian projections may only have a weak participation in vocalisation in the chimpanzees, but in hominids, neighbouring inferior parietal areas were recruited to participate in the planning of motor processes involving vocal articulation. Thus, domain-general ancestral inheritance was accompanied by domain-specific adaptations leading to the development of the arcuate fasciculus capable of supporting complex language processing in humans.

Chapter 2

Maturation of the perisylvian language pathways: effects on lateralisation

2.1 Introduction and summary of findings

In 1865 Paul Broca first proposed the idea of neuroanatomical lateralisation of language. Today, the left hemisphere is regarded as dominant for core language skills, and the left perisylvian brain regions are known to play a quintessential role in language function. However, we still know very little about how development shapes perisylvian language pathways and how it drives the lateralisation of the perisylvian white matter network.

The present study has applied diffusion tensor imaging (DTI) tractography to a group of 101 healthy children, adolescents and adults (age range 9-49 years) to investigate the developmental patterns of the perisylvian language network, and how these affect the resulting anatomical asymmetries. This is a retrospective study that used already acquired data from several projects (IOP brain library), with demographic information limited to age, gender and handedness. This study was largely done before I started my PhD. Processing of data, tractography dissections and data analyses was performed by dr. Marco Catani and dr. Flavio dell'Acqua. I performed the spatial normalisation and created the visitation maps. The study explored whether the maturational changes occurring throughout development are global or involve a specific sub-population of language fibres. By investigating age-related differences in hemispheric lateralisation and the diffusion properties of the white matter in the perisylvian language network several important findings emerged. Firstly, the results showed that individual perisylvian language pathways exhibit distinct maturational trajectories affecting the resulting lateralisation patterns. Frontal lobe connections lateralised very early in life, whereas temporo-parietal connections continued to lateralise and remodel during adolescence and early adulthood, associated with the reorganisation of the white matter connections in the right hemisphere. Developmental changes in the white matter microstructure was evidenced by a significant age-related decrease in the mean diffusivity of all components of the perisylvian language network, except for the long segment in the right hemisphere. The study showed that the development of the perisylvian language pathways continues throughout adolescence and early adulthood with important differences

between genders. It further suggested that language lateralisation is a diverse process, with some tracts already lateralised early in life, whereas others continue to remodel throughout life span.

2.1.1 Brain maturation

One of the most fascinating aspects of the human brain is its dynamic and essentially lifelong development that progressively sculpts the brain to its resulting shape. Although the human brain has around 100 billion neurons at birth, it is only one-quarter of its adult brain volume, due to continuous growth and modifications driven by genes and environment (Toga, et al., 2006). It is currently considered that some periods of life are more critical than others, and many think that the first two decades in life are pivotal for brain development (Yakovlev and Lecours, 1967). However, we must not forget the crucial role of neurogenesis, synaptic pruning and cell shrinkage during the intrauterine period (Rosenzweig, et al., 2012). It has been suggested that these pruning processes occur in order to provide required space and volume to support the later process of myelination (Bartzokis, 2011). These prenatal changes are likely driven by genes, but are also affected by environmental factors such as prenatal stress and nicotine exposure that change the neuroanatomical organisation of the developing brain altering dendritic branching, dendritic length and spine density (Muhammad, et al., 2012; Schwabe, et al., 2012). The postnatal process of myelination is also susceptible to environmental factors. Oligodendrocytes, which are responsible for myelin production, are continually dividing and differentiating and hence epigenetic modifications of gene expression can be introduced in each generation of these cells and reflect environmental conditions at different stages of development (Bartzokis, 2011; Rosenzweig, et al., 2012). Hence, it has been recently accepted that environment can alter the white matter of the brain (Fields, 2008) and recent study provided evidence of experience-related changes in diffusion characteristics in practising pianists (Bengtsson, et al., 2005). Hence, the development of the brain is orchestrated by highly interlinked genetic, epigenetic and environmental mechanisms (see Chapter 4). Both spontaneous brain-specific physiological (Zhou, et al., 2006) and sensory-driven neural activity (environmental) are essential for guiding the process of brain development and the formation, refinement and maturation of synapses (West and Greenberg, 2011). Under environmental stimulation and genetic control, dendritic branching of neurons, increased numbers of synapses, faster conduction speed along fibres due to progressive myelination of the axons followed by the enigmatic process of synapse elimination and dendritic pruning, all lead to more efficient information processing. Exploring the maturation of the brain could bring insights into the cognitive changes during one's life. In order to understand maturation and age-related changes of the perisylvian language pathways, it is necessary to examine closer how the overall brain matures, focusing first on the grey and later white brain matter.

Total brain tissue volume was found to linearly increase from 28 weeks' gestation to term (Rivkin, 2000). Subsequently, cortical thickness and grey matter volume follow a U-shaped developmental course, characterised by a period of initial childhood increase followed by a decline in adolescence (Giedd, et al., 1999; Giorgio, et al., 2010; Gogtay, et al., 2004; Shaw, et al., 2008; Sowell, et al., 2003; Tamnes, et al., 2010). Studies have further observed region-specific patterns of cortical maturation with different areas

developing at different rates and at different times (Giedd, et al., 1999; Gogtay, et al.. 2004; Shaw, et al., 2008; Sowell, et al., 2004; Tamnes, et al., 2010). Cortical regions with simple laminar architecture (3-layered allocortex), including most limbic areas, show simpler developmental trajectories (Shaw, et al., 2008). In contrast, polysensory and high-order association areas of cortex, the most complex areas in terms of their laminar architecture (6-layered isocortex), typically have more complex developmental trajectories (Shaw, et al., 2008). Some of these areas were unique to, or expanded, in primates, lending a possible clue to the developmental trajectories of language-related brain regions. It was found that from ages 5-11 years dendritic pruning and arborisation result in grey matter thinning of 0.15-0.30 mm per year (mostly right dorsal frontal and bilateral parietal regions) whereas in the language areas of temporal and frontal lobes cortical thickening was observed of 0.10-0.15 mm per year (Sowell, et al., 2004). These dynamic but distinct cortical changes in grey matter might be related to the acquisition of complex language abilities after 5 years of age (Sowell, et al., 2004).

In contrast to the nonlinear and regionally specific development of the cerebral cortex, white matter volume has been shown to increase globally in a generally linear manner throughout human life. Both post-mortem histological (Huttenlocher, 1990; Yakovlev and LeCours, 1967) and MRI studies (Giedd, et al., 1999; Paus, et al., 1999; Sowell et al. 2002) note that white matter increases linearly with age in frontal, temporal and parietal regions. This volume increase seems to peak in the fourth or fifth decade of life and then steadily declines due to aging (Sowell et al. 2003). Development of white matter appears to be related to re-arrangement of white matter fibres, increases in the diameter and degree of myelination of the axons forming the fibre tracts, increases in neuronal size and glial proliferation that vary with age at different rates across bundles. Axonal wiring and pruning processes as well myelination, which begins in a scant distribution prior to birth, continue during the postnatal years stretching well into the third decade of life (Rivkin, 2000; Sowell, et al., 2003; Yakovlev and LeCours, 1967). Volpe (1995) has explained the pattern of myelination in human brain as the process that proceeds from proximal pathways to distal pathways – from sensory pathways to motor pathways, from projection pathways to associative pathways, from central loci towards the lobar poles with occipital lobe preceding frontal lobe in completion of the process.

The literature on age-related differences in white matter microstructure, as measured by DTI, shows a consistent pattern of increased fractional anisotropy (FA) and decreased mean diffusivity (MD) during childhood, adolescence, and even early adulthood (Ashtari et al., 2007a; Barnea-Goraly et al., 2005; Eluvathingal et al., 2007; Giorgio et al., 2008; Lebel et al., 2008; Nagy et al., 2004; Schmithorst et al., 2002; Zhang, et al., 2007), but after the fifth decade of life FA steadily decreases due to aging (Voineskos, et al., 2012). The full maturity for all white matter tracts but one (cortico-spinal tract) is reached during the 3rd and 4th decades of life (23.1-39.4 years) as measured by FA (Kochunov, et al., 2012). With increasing maturity of the brain, there is less motion of water molecules, since the extracellular space is diminished because of the proliferation of myelin and the increased size of maturing neurons and glia, resulting in a decrease in MD. In addition, the motion of the water molecules becomes increasingly anisotropic, due to a hinderance of water motion by the myelin sheath, oligodendrocytes, intraaxonal macromolecules and functional ionic channels, all leading to FA increase (Barkovich, 2000). Lebel et al. (2008) reported age-related increases in

FA and decreases in MD in major white matter tracts (including the superior longitudinal fasciculus) and selected subcortical regions in the age span 5-29 years. Studies suggest that this early age-related increase in FA in tracts is primarily driven by a reduction in perpendicular diffusion (Dperp), whereas parallel diffusion (Dpar) remains relatively stable or decreases slightly across different age groups (Asato, et al.,2010; Giorgio et al. 2008; Lebel et al. 2008). Still, others have reported a reduction of both Dperp and Dpar in many regions but usually to a greater extent in Dperp (Eluvathingal et al. 2007; but see Ashtari et al. 2007a). Investigating white matter maturation is important since recent diffusion imaging studies point to an association between structural maturation of neural pathways (when using FA as an index of white matter maturation) and the successful development of cognitive functions (Paus, 2010). Several groups have reported a positive relationship between various cognitive skills and FA in different tracts, such as the arcuate fasciculus (Ashtari, et al., 2007a,b), corpus callosum (Fryer et al., 2008; Muetzel et al., 2008; Nagy, et al., 2004) and other multiple white matter tracts (Schmithorst, et al., 2005).

How are grey matter and white matter maturation trajectories related? Are the loss ('pruning') and gain (intra-cortical myelination) of tissue, which can be observed around puberty, related? Tamnes, et al. (2010) found that cortical age-related thinning was not explained by white matter maturation (volume increases, and changes in diffusion parameters) in one-hundred and sixty-eight participants aged 8-30 years. Only moderate associations between cortical thickness and both volume and diffusion parameters in underlying white matter regions were found, but the relationships were not strong. However, most of these DTI studies have been cross-sectional, further highlighting the importance of longitudinal studies that would enable the true reconstruction of the dynamic course of the developing brain (Toga et al., 2006) and correlation with behavioural outcomes.

After the process of brain maturation the steady decline is observed, known as brain aging or atrophy. Brain atrophy accelerates with increasing age and no gender difference is found in the rate of brain atrophy (Takao, et al., 2012). Age-dependent neuronal loss has long been considered central to age-related decline, associated with oxidative damage to the RNA molecules (Nunomura, et al., 2012) and iron accumulation which increases the toxicity of environmental or endogenous toxins (Zecca, et al., 2004). However, recently, age-related changes in brain white matter have taken precedence in explaining the steady decline in cognitive domains seen in the elderly (Hinman and Abraham, et al., 2007). Using a newly developed stereologic method it was found that males have a staggering total myelinated fibre length of 176,000 km at the age of 20, but only 97,000 km at the age of 80, whereas the total length in females was 149,000 km at the age of 20 and 82, 000 km at the age of 80. This finding corresponds to a 10% decrease per decade in myelinated nerve fibres (Marner, et al., 2003), and could explain cognitive decline observed in the elderly. In the normal aging population Voineskos, et al. (2012) found that a decrease in FA of white matter fibres correlated with cognitive decline: inferior longitudinal fasciculus (ILF) with visuomotor dexterity; the inferior occipitofrontal fasciculus with visuospatial construction; and posterior fibres (i.e. splenium) of the corpus callosum with memory and executive function. A general pattern emerged indicating that the prefrontal white matter is most susceptible to the influence of age (Gunning-Dixon, et al., 2009). Nevertheless, structural imaging studies showed that active cognitive lifestyle may be protective against development of brain

atrophy in late life (Suo, et al., 2012). Enriched environment of physical, social and sensory stimuli, can alleviate the normal aging process of the white matter marked by demyelination and loss of oligodendrocytes, and associated with cognitive decline (e.g. in spatial memory) (Yang, et al., 2012). Enriched environment can recover cognitive functions and promote remyelination in the aged brain. DTI tractography offers a valid approach to study the white matter maturation and age-related effects, as measured by FA and MD, and probe the associations with various cognitive functions.

It is important to note that significant gender differences are observed during brain maturation. Sexual dimorphism was found for both global and regional grey and white matter development. Studies of children and adolescents showed different ages of volume peak for cortical and subcortical grey matter in males and females (Giedd, et al., 1996, 1999; Lenroot and Giedd, 2006), with rates of brain maturation higher for females. EEG studies supported this and showed that regions involved in language (e.g. Wernicke's and Broca's area) and fine motor skills mature significantly earlier in girls than in boys (Anokhin, et al., 2000; Hanlon, et al., 1999). Sexually dimorphic developmental patterns are observed for white matter, i.e. the arcuate fasciculus where FA increased in girls but decreased in boys during childhood (Schmithorst, et al., 2008). Studies further found steeper growth of white matter during childhood (Lenroot, et al., 2007; Rosenzweig, et al., 2012) and consequent FA decline in adulthood (Rosenzweig, et al., 2012) in males compared to females. These gender differences might be driving the differences in the incidence of neuropsychiatric disorders, with females being at reduced risk of developing some disorders like autism (Baird, et al., 2006) and at greater risk of developing others, like affective disorders (Kessler, et al., 1994) and Alzheimer's disease (Andersen, et al., 1999). The important question is which factors are driving these gender differences during brain development. A number of biological mechanisms have been suggested that might provide explanations. Animal and human studies show that sex hormones are important for development of sexual dimorphism during early brain maturation, especially the effects of testosterone (Perrin, et al., 2008, 2009; Phoenix, et al., 1959) and oestrogen (Kochunov, et al., 2012), which has neuroprotective effects and increases the number of neurons, branching complexity and dendritic spine density in animal models (Brinton, 2001; Hao, et al., 2006; Sandstrom and Williams, 2001). Furthermore, sex hormones can modulate brain function, and previously hormonal variation across the menstrual cycle was associated with significant changes in brain activity during language tasks (Dietrich, et al., 2001; Fernandez, et al., 2003; Goldstein, et al., 2005). However, sexual dimorphisms are also driven by factors other than sex hormones. Animal studies observed significant gender differences in gene expression levels even prior to the development of gonads (Dewing, et al., 2003) suggesting that gender differences in the brain may be present prior to and independent of the sex hormones. Sex chromosomes are other important culprits in driving these differences during brain development. The effects of sex chromosomes are studied in relation to Turner's syndrome, a genetic disorder in females where normal second X chromosome is lacking. Findings note that the mode of inheritance of the X chromosome affects the development of specific brain regions. Observed X chromosome monogamy alters global and regional brain volumes (Cutter, et al., 2006), and more importantly alters language system in the temporal and frontal lobes (Temple and Shephard, 2012). Hence, it is likely that this present diffusion tractography study will observe significant gender differences in the maturation of the perisylvian language network.

2.1.2 Language maturation

Language functional maturation

Prior studies of maturation of language function support the widely held view that language develops continuously over time. Discontinuities may sometimes be observed in children with language impairments. Electrophysiological and hemodynamic studies indicate that although language functional lateralisation is present at birth, phonological processes appear during the first months of life, semantic processes at 12 months, and syntactic processes around 30 months (Friederici, 2006b).

First exposure to language is based on acoustic and phonological/phonetic information, and therefore the initial step for every infant is to discriminate between speech and non speech sounds. Even in the first days after birth newborns are able to discriminate between different phonemes and distinguish the sentence prosody of their mother tongue from that of other languages (Friederici, 2006b). Sleeping newborns showed a larger increase in cerebral blood volume over the left temporal brain regions for forward speech compared to backward played speech in an optical imaging experiment (Pena et al., 2003). However, the neuroanatomical basis of these early abilities still needs to be fully specified. An fMRI experiment with 3-month-olds suggests that at this age language processing is supported by inferior frontal and temporal brain regions similar to adults, with stronger left hemispheric activation of the superior temporal gyrus for speech sounds as measured by forward and backward speech compared to silence (Dehaene-Lambertz et al., 2002). The same study also found differences between forward and backward speech in the left angular gyrus and precuneus. This is in line with other fMRI studies suggesting that neonates and 3-months old infants recruit areas beyond temporal lobes: the inferior and dorsolateral frontal regions when engaged in a speech task (Dehaene-Lambertz et al., 2002, 2006; Bristow et al, 2009) and the anterior prefrontal cortex when social cues are present (Dehaene-Lambertz et al., 2010). Findings on the participation of frontal lobes came as a surprise since they used to be neglected in previous studies of early language processing, due to their protracted maturational course. However, recently Leroy et al. (2011) showed that even at this early stage in life frontal maturation is sufficient for functional language activity (Leroy, et al., 2011). Thus, many studies reported early left hemispheric dominance for speech recruiting multiple brain regions, similar to that of adults. Nevertheless, Perani, et al. (2011) observed that in 2-days old infants, language-related neural substrate is fully active in both hemispheres with a preponderance in the right auditory cortex. However, functional and structural connectivities within this neural network were still immature, with strong connectivity only between the two hemispheres, in contrast to the adult pattern of dominant intrahemispheric connectivities (Perani, et al., 2011).

Recent data also indicate that language-specific neural representations of words form as early as 4 months of age in the infant brain (Friederici, et al., 2007) and by 9 months of age infants have already acquired the inventory of phonemes and stress patterns of their mother tongue (Jusczyk, 1997). They start to produce their first words between 11 and 13 months, with a lexicon gradually increasing from 50 to 75 items by the age of 16 months to a proper vocabulary between the age of 18 and 24 months (Bates and Goodman, 1999).

In the second and third year of life, infants acquire syntactic structures and the first production of two-word utterances, and later more-word utterances (Friederici, et al., 2006b; Hohle et al., 2001). It has also been reported that 14- to 20-month-olds are already able to differentiate between familiar and unfamiliar words (Mills et al., 2004), however it was not clear whether infants at this age process the semantics of words in a similar fashion to adults. What is known is that in early childhood event-related potentials (ERPs) differ as a function of word meaning, and their distribution change from bilateral at 13 months to left hemisphere dominant at 20 months of age during language comprehension (Mills et al., 1997).

Imaging studies point to the increasing functional lateralisation during childhood and adolescence (Everts et al., 2009; Holland et al., 2007; Mills et al., 1997; Ressel et al., 2008; Szaflarski et al, 2006) indicating a functional reorganisation of the neural network underlying language towards a left lateralised language system. Some authors suggest that these functional changes correspond to language skill acquisition rather than global brain maturation (Holland et al., 2007). A recent fMRI study by Friederici, et al. (2011) showed that in contrast to adults who show strong effective connectivities between frontal and temporal language regions within the left hemisphere, six-year-old children's default language network is characterised by stronger functional interhemispheric connectivity, mainly between the superior temporal regions. The observed pattern in children is in line with other fMRI studies that report children's stronger reliance on the right hemisphere, reflected in a more right functional lateralisation during language processing as compared to adults (Brauer and Friederici, 2007; Brauer, et al., 2008). In contrast with the left hemispheric perisylvian cortex that supports the processing of semantic and syntactic information (Friederici, 2002), the right perisylvian cortex is responsible for processing prosodic information (Meyer et al., 2002, 2004). Thus, it has been suggested that the stronger reliance of the right hemisphere in children is due to a higher involvement of prosodic processes in language processing (Sabisch, et al., 2009; Brauer, et al., 2008). This observed difference between children and adults is in line with the assumption of ongoing structural maturation of the perisylvian brain regions and the connections between them.

From the age of 5- to 10-years, language processing in children, observed using semantic categorization tasks and fMRI, has a similar activation pattern to those of adults (activation of the frontal and temporal regions of the left hemisphere) suggesting that language is left-lateralised as early as 5 years of age (Balsamo, et al., 2006). However, development of language functions is an on-going process, and behavioural data indicate that the processing of syntactically complex sentences, such as sentences with a noncanonical word order that do not follow the usual subject-verb-object word order, occurs late. From fMRI data in adults, we know that to process the noncanonical sentences we recruit Broca's area (Stromswold, 1996; Rogalsky, et al., 2008) and the posterior superior temporal gyrus/superior temporal sulcus (Bornkessel, et al., 2005; Friederici, Fiebach, et al., 2006), areas which are connected by the arcuate fasciculus (Catani, et al., 2005). It is hence possible that the processing of complex syntax coincides with the maturation of the arcuate fasciculus fibre bundle. Other complex language processing that occurs late includes understanding object-first sentences (Dittmar, et al., 2008) or passive sentences (Hahne, et al., 2004) which occurs around the age of 7 years.

In order to explore the potential influence of variation in early language experience on shaping of brain function and structure and its potential for plasticity, many studies turned towards bilingualism (Hull and Vaid, 2007, 2006; Golestani and Pallier, 2006; Mechelli, et al., 2004; Gandour, et al., 2007). Recent discoveries suggest that bilingual experience may confer unique patterns of neurofunctional activity. After two meta-analyses of 66 behavioural studies, Hull and Vaid (2007) showed that monolinguals and late bilinguals (second language acquired after the age of six) in general have left functional hemispheric dominance across language tasks regardless of proficiency, whereas early bilinguals (second language acquired before the age of six) have bilateral hemispheric involvement. Corballis (1991) suggested that language might be left lateralised because the left hemisphere develops more rapidly than the right during early development. However, these findings suggest that left-lateralisation may be a consequence of housing only one language system, and the speculation is whether the right hemisphere could similarly undergo rapid early growth when multiple languages must be accommodated. A voxel-based morphometry study (Mechelli, et al., 2004) showed that brain structure is affected by bilingual experience, since grey matter density increases in bilinguals over monolinguals in inferior parietal cortex. The effect was greater in the early bilinguals in the left and right hemisphere. The density in this region increased with the second language proficiency, but decreased as the age of acquisition increases. This relationship between grey matter density and proficiency/age of acquisition suggests that the attainment of better skills in a second language or earlier learning of second language results in structural reorganisation in this language region.

Little is known how functional language maturation is related to the underlying neural organisation of the perisylvian language network. The next section will discuss the maturation of the arcuate fasciculus in order to provide a relevant background to the present diffusion tractography study.

Language structural maturation: arcuate fasciculus

The fibres of the arcuate fasciculus are among the slowest ones to mature in the human brain (Paus, 1999), and imaging-based anatomical data suggest that its maturation is associated with different structural changes.

Converging evidence from fMRI and DTI indicate that the arcuate fasciculus is still immature in 7-year old children (as measured by diffusion anisotropy); and although children make use of this dorsal pathway, it is suggested that due to its relative immaturity they extend their fronto-temporal network by making additional use of a ventral extreme capsule fibre system (Brauer, et al., 2011). Hence, it is suggested that adults make use of a more confined language network compared to children, due to the ongoing maturation of this structural network. As Annette Karmiloff-Smith (2010) wrote, neural processing tends initially to be diffused across both hemispheres, but with developmental time and the continuous processing, brain activity becomes increasingly restricted to more specific networks. Continuous maturation is further highlighted by highly significant changes of fibre orientation in regions which correspond to the superior longitudinal (arcuate) fasciculus during the first 5 years of life (Zhang, et al., 2007). This late maturation of the arcuate

fasciculus could be explained in terms of progressive axonal reorganisation and late myelination, due to increasingly complex use of this network in speech and language processes.

Using DTI, even in weakly myelinated brains the main fibre tracts can be identifiable and thus, maturational changes of language network can be observed (Dubois, et al., 2006, 2009). Dubois et al, (2009) studied in-vivo structural markers of hemispheric language asymmetries in infants from 1-4 months of age and found left-right differences in the arcuate fasciculus during the first post natal weeks, with no evolution in the amplitude of these differences at later stages (3.9 - 18.4 weeks). However, this study did not manage to reconstruct consistently the frontal portion of the tract, because of insufficient diffusion anisotropy in this age range, so only two segments, temporal and parietal, were analysed. Nevertheless, the results indicated distinct developmental patterns for different segments of the arcuate fasciculus. Analysis on localisation, geometry and diffusion indices revealed an asymmetry in the temporal segment of the arcuate fasciculus. At this age the temporal segment of the arcuate fasciculus is already larger in the left hemisphere as shown by voxel-based analysis, and exhibits higher FA, which may imply higher coherence of fibres; and higher MD and longitudinal diffusivity – suggesting a delayed "pre-myelination" stage, compared to its right counterpart. Further, maturation seemed more advanced in the left parietal segment of the arcuate fasciculus compared to its right counterpart, as measured by FA. Authors suggested that the higher FA observed in the left parietal segment is related to a higher organisation of parallel fibres, rather than attributing it to advances of "true" myelination (corresponding to the sheathing of oligodendroglial processes around the axons), as this region matures slowly during childhood. These early left asymmetries suggest that structural language lateralisation might be related to the functional lateralisation of language.

Diffusion imaging studies suggest that the arcuate fasciculus exhibits changing fibre coherence and/or fibre density during development. Age-related increases in FA were found for both left and right arcuate fasciculus (Ashtari et al., 2007a; Bonekamp et al., 2006; Eluvathingal et al., 2007; Schmithorst et al., 2002). Barnea-Goraly et al. (2005) observed age related increases in FA and fibre density in intrahemispheric tracts which correspond to the location of long segment of the arcuate bundle during childhood and adolescence. Similarly, Schmithorst et al. (2002) found significant positive correlation of FA and significant negative correlation of the apparent diffusion coefficient with age in the left arcuate fasciculus for both children and adolescents. These age-related increases in fractional anisotropy could be compatible with a mechanism of an increasingly dense and ordered packing of fibre tracts, which leads to directionally more hindered extracellular, rather than intracellular space. It is however unclear whether increases in FA are due to greater diffusivity along the main diffusion axis (Ashtari et al., 2007a), reduced diffusivity along the axes perpendicular to it (Bonekamp et al., 2006; Eluvathingal et al., 2007; Giorgio et al., 2008; Snook et al., 2005; Suzuki et al., 2003) or a combination of the two. Giorgio, et al. (2010) showed an age-related increase in FA of the arcuate fasciculus driven by increases in parallel diffusivity during adolescence (in line with Ashtari, et al., 2007a). FA values of both left and right arcuate fasciculus were significantly higher at the end of the follow-up compared to baseline. The authors found an overlap between age-related FA and white matter volume increase at the level of the bilateral superior longitudinal fasciculi (including arcuate fasciculi). Taken together, these age-related differences probably reflected the increases in the diameter of the axons of the

arcuate fasciculus in both hemispheres. However, Paus et al. (1999) revealed significant age related increases in white matter density in the posterior portion of the left, but not right, arcuate fasciculus. Moore (2002) examined brain specimens of children, and observed gradual maturation of axons originating in the superficial layers of the auditory cortex; these axons possibly contributed to cortico-cortical connections contained within the arcuate fasciculus. Thus, author argued that age-related increases in white matter density along the arcuate fasciculus may represent a structural correlate of another component of the auditory-vocal system, namely the cortico-cortical pathway mediating sensory-motor interactions between the anterior and posterior speech regions. It is also possible that age-related increases in white matter density reflect the effect of extensive use of this system during one's life. Taken together, these age-related changes in FA and fibre density, mainly observed along the left arcuate fasciculus, may reflect increases in axon diameter, myelination, fibre coherence, axonal membrane integrity, separately or in combination. However, we cannot conclude directly which cellular changes are involved in the dynamic maturational processes, because MRI lacks the resolution to characterise the exact cellular mechanisms.

The first study to propose that maturation of the arcuate fasciculus might not be uniform for its three segments was done by Eluvathingal et al. (2007). The authors studied the effects of age, and lateral asymmetries in right-handed children using DTI, and noticed that different arcuate fasciculus segments showed different patterns of lateralisation. The long direct segment exhibited higher FA in the left hemisphere, while the anterior indirect segment showed a significant right asymmetry in the FA, consistent with the findings of Buchel et al. (2004). The authors suggested that the less prominent or absent long segment of the arcuate fasciculus on the right side (observed in 29% of participants) allowed the segment to be straighter on the left side, leading to the observed differences in diffusivity parameters. These differences in lateralisation can lead to a conclusion that different segments may have different maturational trajectories, and that the observed lateralisation differences merely reflect different stages of white matter maturation. Eluvathingal et al. (2007) proposed two different patterns of white matter maturation, affecting differently on one hand the anterior indirect segment and on the other the long direct and posterior indirect segments. First pattern of white matter maturation observed for the bilateral anterior segment was characterised by a significant increase in FA and a decrease in all three diffusivities (mean, transverse and axial) suggesting that myelination (as measured by decreased transverse diffusivity) is accompanied by changes in the intrinsic characteristics of axons/changes in extra-axonal or extracellular space (decrease in axial diffusivity). The authors suggested that this pattern of maturation plays an important role in higher cognitive functions. Myelination in general is known to coincide with the development of various cognitive skills (Yakovlev and Lecours, 1967; Mabbott, et al., 2006; Nagy, et al., 2004), such as language-related reading (Kraft, et al., 1980), and development of vocabulary (Pujol, et al., 2006). On the other hand, significant age-related decrease in all three diffusivities not accompanied by significant increase in FA was observed in the left long segment and bilateral posterior indirect segment of the arcuate fasciculus. Although this may be attributable to progressive myelination, the changes in the axial diffusivities may also indicate continued changes in intrinsic characteristics of axons/changes in extracellular space. Whether different maturational trajectories of the three segments influence the resulting lateralisation patterns and whether language anatomical asymmetry changes across lifespan will be discussed in more detail in the following section.

2.1.3 Age-related differences in lateralisation patterns

Earlier reports confirmed dominant left asymmetry of the arcuate fasciculus by microscopic examination of post-mortem specimens (Galuske, et al.,2000) structural MRI (Paus, et al., 1999) and DT-MRI (Catani, et al., 2005; Eluvathingal, et al., 2007; Hagmann, et al., 2006; Nucifora, et al., 2005; Parker, et al., 2005; Powell, et al., 2006; Vernooij, et al., 2007) for both volumetric (Hagmann, et al., 2006; Parker, et al., 2005; Paus, et al., 1999) and diffusion parameters (Eluvathingal, et al., 2007; Nucifora, et al., 2005; Powell, et al., 2006; Vernooij, et al., 2007). However, not all studies found a left-sided asymmetry of the arcuate fasciculus. Giorgio et al. (2010) observed that FA values computed from the whole arcuate fasciculus by probabilistic tractography were significantly higher in the right compared to the left hemisphere at different time points during adolescence. This was not explained by differences in segmented tract volumes and appeared to conflict with previous cross-sectional brain asymmetry DTI studies in children, adolescents (Eluvathingal, et al., 2007) and adults (Buchel, et al., 2004; Catani, et al., 2005). However, the authors suggested that these differences reflected different biases in localisation of changes. In two of the aforementioned studies (Buchel et al., 2004; Eluvathingal et al., 2007) a right-sided FA asymmetry was found in the fronto-parietal (anterior) segment of the arcuate fasciculus, which might be leading the right-sided asymmetry of the results obtained by Giorgio et al. (2010). This is likely considering that previous diffusion tractography studies revealed a highly heterogeneous distribution of the degree of lateralisation of the three segments of the arcuate fasciculus in the human population (Catani, et al., 2007; Eluvathingal, et al., 2007). In addition, significant differences were observed between genders, with females more likely to have a symmetrical bilateral pattern than males of the long direct segment. This symmetrical distribution of the long segment was correlated with better performances in complex verbal memory tasks (Catani, et al., 2007). This might not be true for other measures of language function, since extreme left-sided asymmetry of the long segment was associated with better receptive vocabulary scores, while a phonological processing task was performed best by those with more moderate left-sided lateralisation (Lebel and Beaulieu, 2009). Although it is not clear from these recent studies how lateralisation of the arcuate fasciculus affects different language skills, it is certain that it plays an important role in cognitive language-related tasks.

Having in mind the association between language anatomical lateralisation and cognitive skills, it is important to investigate whether, and how, language lateralisation changes across the lifespan. Lenneberg (1967) proposed that language could be acquired only during a critical developmental period during childhood. In his "plasticity hypothesis" he argues that both maturational and environmental factors lead to a gradual specialization of the left hemisphere for language, which is completed around puberty. This would imply that language lateralisation is a dynamic process that continues until the beginning of adolescence. However, this hypothesis has been challenged by imaging findings reporting that a degree of left hemisphere lateralisation exists shortly after birth (Dubois, et al., 2009). The period of brain plasticity was originally assumed to be associated with incomplete brain lateralisation, and subsequently identified in the literature with the ongoing processes of myelination, dendritic pruning, creation of neural networks and with higher levels of glucose uptake (Snow, 2002). Contrary to the classical critical period, a newer concept of the

'optimal period' refers to a window that is more variable in onset and offset, and supports the language hypothesis of one or several optimal periods in language acquisition (Werker and Tees, 2005).

Structural imaging studies also brought a wealth of evidence suggesting that the left asymmetry of the arcuate fasciculus is stable, and present very early in human life. This might seem surprising considering that the arcuate fasciculus matures relatively late in life (Brauer, et al., 2011; Giorgio et al. 2008). Nevertheless, research points to the fact that in general brain asymmetries occur very early in life. Hence, left-right asymmetry becomes evident in the early human embryonic stages, driven by asymmetrical gene expression in the perisylvian cortical regions (Abrahams et al., 2007; Sun et al., 2005). Gene expression asymmetries of the perisylvian cortex seem to mirror the asymmetries of language functional and anatomical organisation, favouring the left hemisphere. Further, if we investigate the language anatomical lateralisation by studying the asymmetry of the planum temporale (PT), which overlaps partly with Wernicke's area, we can observe this left PT asymmetry in fetal brains as early as 29-31 weeks of gestation (Wada, et al., 1975). Lastly, DT-MRI studies point to left asymmetry of the arcuate fasciculus in the first months of human postnatal life (Dubois, et al., 2009) and childhood (Eluvathingal, et al., 2007; Lebel and Beaulieu, 2009). These asymmetric structural changes of the language network are probably linked to an early language functional asymmetry (Dehaene-Lambertz, 2000; Dehaene-Lambertz et al., 2002; 2006; 2010; Pena et al., 2003). Lebel and Beaulieu (2009) reported a consistent pattern of left asymmetry of the arcuate fasciculus in children, adolescents and young adults, stable across age and gender, and concluded that arcuate fasciculus lateralisation seems to be constant throughout human life. Nevertheless, it is not known whether this stable and early lateralisation is present for all three segments of the arcuate fasciculus, and this will be investigated in the present PhD study. Also, stable anatomical lateralisation does not imply stable functional lateralisation of language. Although structural studies observed constant lateralisation pattern, which does not change from early childhood into adulthood, functional studies note an age-related increase in the degree of lateralisation favouring the left hemisphere during language processing tasks (Brauer and Friederici, 2007; Holland, et al., 2007; Perani, et al., 2011; Szaflarski, et al., 2006). Yet, no study to date has separately examined the maturation of the three segments of the arcuate fasciculus, and their hemispheric asymmetries over time. Hence it is not understood whether the maturational changes occurring throughout development are global or involve a specific sub-population of language fibres. Having different maturational trajectories might imply different functional specialisations of the three segments. That asymmetries of different segments might have different maturational patterns could be expected if we consider the maturation of grey matter overlying the language network. For example Leroy, et al. (2011) analysed asymmetries in Broca's regions, and found that BA44 (pars opercularis) is significantly more asymmetric towards the left than area BA45 (pars triangularis), suggesting a developmental gradient in asymmetry that begins in BA44 and extends progressively towards BA45. This is in line with a study by Amunts, et al. (1995) who reported that asymmetry in BA45 gray matter thickness increased with age, while it was already established in BA44 even in young infants. Likewise, it is possible that the three segments of the arcuate fasciculus have different maturational trajectories reflected in resulting asymmetry differences. Thus my aim was to decipher age-related differences in the fronto-temporo-parietal perisylvian network underlying language processing, and elucidate the effects of maturation on lateralisation patterns of fibre bundles running within the arcuate fasciculus.

2.2 Methods

Study Participants

We recruited 101 right-handed healthy volunteers between the age of 9 and 49 years. There was almost equal number of females and males included (50 males, 51 females). Handedness was assessed by using the Edinburgh Handedness Inventory (Oldfield, 1971). Approval was obtained from the Joint Medical Ethical Committee of the Institute of Psychiatry, Kings College London. Informed written consent was obtained from all participants.

DT-MRI Acquisition and Processing

Data was acquired on a GE Signa 1.5-T LX MRI system (General Electric, Milwaukee, WI) with 40-mT/m gradients, using an acquisition sequence fully optimised for DT-MRI of white matter, providing isotropic resolution (2.5 x 2.5 x 2.5 mm) and coverage of the whole head. This acquisition was gated to the cardiac cycle using a peripheral gating device placed on the subjects' forefingers. There were 64 uniformly distributed directions used, with 7 b0 images, with b-value being 1300 s/mm^{-2}. Full details of the acquisition sequence are provided in Jones et al. (2002). DTI acquisition per subject took approximatively 15/20 min according to the heart rate. Following correction for the image distortions introduced by the application of the diffusion encoding gradients, the DT was determined in each voxel following the method of Basser et al. (1994). After diagonalisation of the DT, different quantitative indices were estimated in each voxel, e.g. fractional anisotropy and mean diffusivity. To ensure that the observer was blind to the hemisphere during virtual dissection of the language pathways and to provide protection against subjective bias, some of the anonymised DT-MRI datasets were flipped about the midline.

Tractography Algorithm and ROI delineation

Tractography was performed using in house software and was based on the procedure originally described by Basser et al. (2000). Details of the method were published before (Catani, et al., 2005), but a brief description will follow. Firstly, a continuous description of the DT field was obtained using a B-spline fitting on the elements of the tensor from each voxel (Basser, 2004). This procedure allows rapid evaluation of the DT at any arbitrary location within the imaged. The regions of interest were selected, and the voxel inside these regions considered as the starting point of the tractography (“seed points”). For each seed point we propagated the streamline following the directions of the principal eigenvector. The track was propagated by 0.5mm step along this direction. The DT was then determined at this new location and the orientation of its principal eigenvector estimated. The procedure was repeated iteratively. A pathway was tracked until the fractional anisotropy of the tensor was below a fixed arbitrary threshold (0.2) or the curvature was less than 30 degrees. The procedure was then repeated by tracking in the opposite direction, to reconstruct the whole tract passing through the seed-point.

ROI delineation method

A two regions of interest (ROI) approach described in Catani et al. (2005, 2007) has been used in this study to dissect the three segments of the perisylvian pathways (see Fig 2.2.1). ROIs were defined on the axial fractional anisotropy maps, to encompass the horizontal fibres lateral to the corona radiata and medial to the

cortex extending from Talairach z=22 to z=28. All fibres passing through this ROI were reconstructed in three dimensions using MATLAB (Mathworks, Natick, MA) and visualised as illuminated streamtubes. The ROI was defined on axial slices as this projection facilitates the visualisation of the borders between the fibres of the arcuate and those of the corona radiata. A two-ROI approach was used to perform further detailed dissection of the arcuate fasciculus, allowing separation of different sets of fibres within the arcuate bundle. Two spatially separated regions are defined in the fractional anisotropy volume, and all fibres passing through both are visualised. The approach does not constrain the tracts to start and end within the defined regions, only to pass through them. The distribution of the arcuate fasciculus terminations found by tractography extends beyond the classical limits of Broca's and Wernicke's areas to include, in addition to the inferior frontal cortex, part of the middle frontal gyrus and, in addition to the superior temporal cortex, the posterior middle temporal gyrus respectively. For this reason, these regions are referred to as Broca's and Wernicke's territories. The single segments were visually inspected for the presence of aberrant streamlines and anatomical correspondence between the two hemispheres. The number of seeds used to start tracking was similar between the two hemispheres.

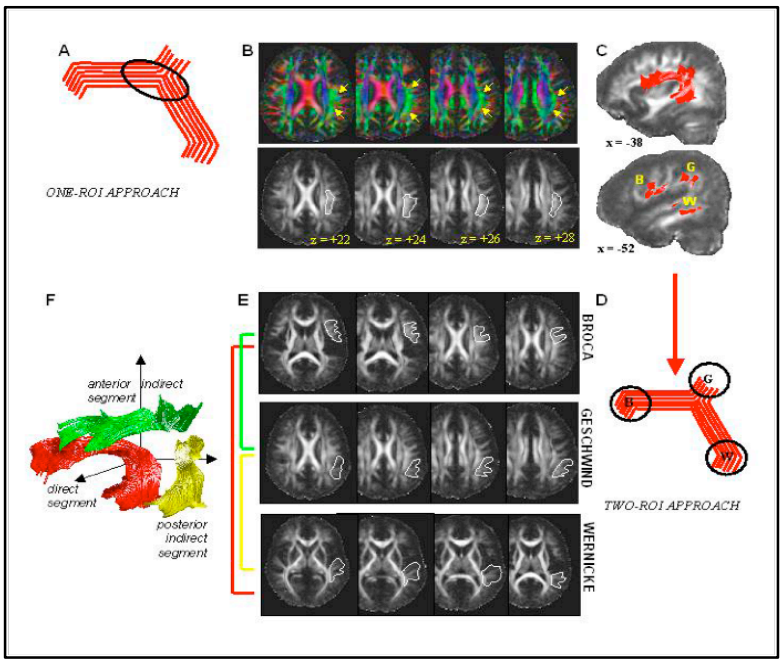

Fig 2.2.1 (adapted from Catani, et al., 2005) Virtual dissections of perisylvian language pathways using one- and two-region of interest approaches. (*A*) A large region of interest (ROI) is defined around the central part of the arcuate fasciculus. (*B*) Guided by the colour-encoded fibre orientation map on the upper row, where the green fibres of the arcuate fasciculus (indicated by the yellow arrows) pass lateral to the corona radiata (blue), a region of interest (encircled in white) is defined through four axial fractional anisotropy images (lower row). (*C*) Pathways passing through the ROI are displayed in red and superimposed on sagittal fractional anisotropy images. The most lateral image (Talairach x -52) shows the three cortical projection territories of the arcuate fasciculus: posterior inferior frontal territory (B, Broca's territory), inferior parietal territory (G, Geschwind's territory) and superior posterior temporal region (W, Wernicke's territory). (*D*) A two-region of interest approach is used to dissect connections between the Broca's, Geschwind's and Wernicke's territory. (*E*) ROIs are defined on axial fractional anisotropy images. (*F*) Connections from Broca's to Geschwind's territory are displayed in green (anterior indirect segment), connections from Wernicke's to Broca's territory in red (long direct segment) and connections from Wernicke's to Geschwind's territory in yellow (posterior indirect segment).

At the termination of tracking, the number of reconstructed pathways, the fractional anisotropy, which quantifies the directionality of diffusion on a scale from zero (when diffusion is totally random) to one (when water molecules are able to diffuse along one direction only) and mean diffusivity were sampled at regular (0.5mm) intervals along the tract and the means computed. For each reconstructed segment a lateralisation index was calculated counting the number of reconstructed pathways within each hemisphere. Lateralisation index was obtained according to the following formula (N., number):

$$\frac{\text{(N. streamlines-left)} - \text{(N. streamlines-right)}}{\text{(N. streamlines-left)} + \text{(N. streamlines-right)}]/2}$$

Positive values of the index indicate a greater number of streamlines in the left segment compared with the right. Values around the zero indicate a similar number of streamlines between left and right. Similarly, a lateralisation index was calculated for the fractional anisotropy of each segment.

Visitation Maps and Tract Volume Measurements

All the tracts were first converted into binary maps. We used SPM5 normalisation pipeline, which is affine normalisation followed by non-linear. Binarisation occurred after normalisation to the native space. Binary maps with dimensions equal to that of the DT-MRI data (i.e., 128 x 128 x 60) were computed by assigning each voxel a value of 1 or 0 depending on whether the pixel was intersected by the tract segment or not. All the binary masks were normalized to the MNI space (defined by the Montreal Neurological Institute). Normalization of all the brains first required an adequate template to be estimated. The b0 images from 60 subjects were then spatially normalized to the MNI reference space defined by the Echo Planar Imaging (EPI) template supplied as part of the SPM5 software package (statistical parametric mapping; Wellcome Department of Cognitive Neurology, London, U.K.). The same transformation was applied to the fractional anisotropy (FA) maps for each subject. FA maps were then averaged, and the result was regarded as the first guess template. In order to refine and improve the quality of this first template, we repeated the normalization of the original FA maps but this time using the guess as the new template. Afterwards all the FA maps were averaged to obtain the final template, and on this final template the normalization of FA maps was repeated. The final transformation was applied also to the binary maps produced for each subject and segment. All the datasets were sorted according to age. A moving average technique with a square window of ±5 years was applied, and an average visitation map for each year ± the width of the window was obtained. There were approximately the same number of participants in each windon (around 10 subjects). Visitation map is defined as the average of the binary maps inside the windows, where the voxel value of 1 is reached when all the subjects present streamline in that voxel or 0 when no streamlines are present in the given voxel. This technique enabled us to investigate and follow the volume changes of different segments of the tract at different ages.

2.3 Results

Age-related differences in language lateralisation

The following are the analyses previously done by the members of the Natbrainlab, dr. Marco Catani, dr.Flavio Dell'Acqua and dr. Luca Pugliese. For each segment of the arcuate fasciculus the number of streamlines in both hemispheres was counted and a laterality index was calculated. Variations in the laterality index of the three segments were plotted against age (Fig 2.3.1).

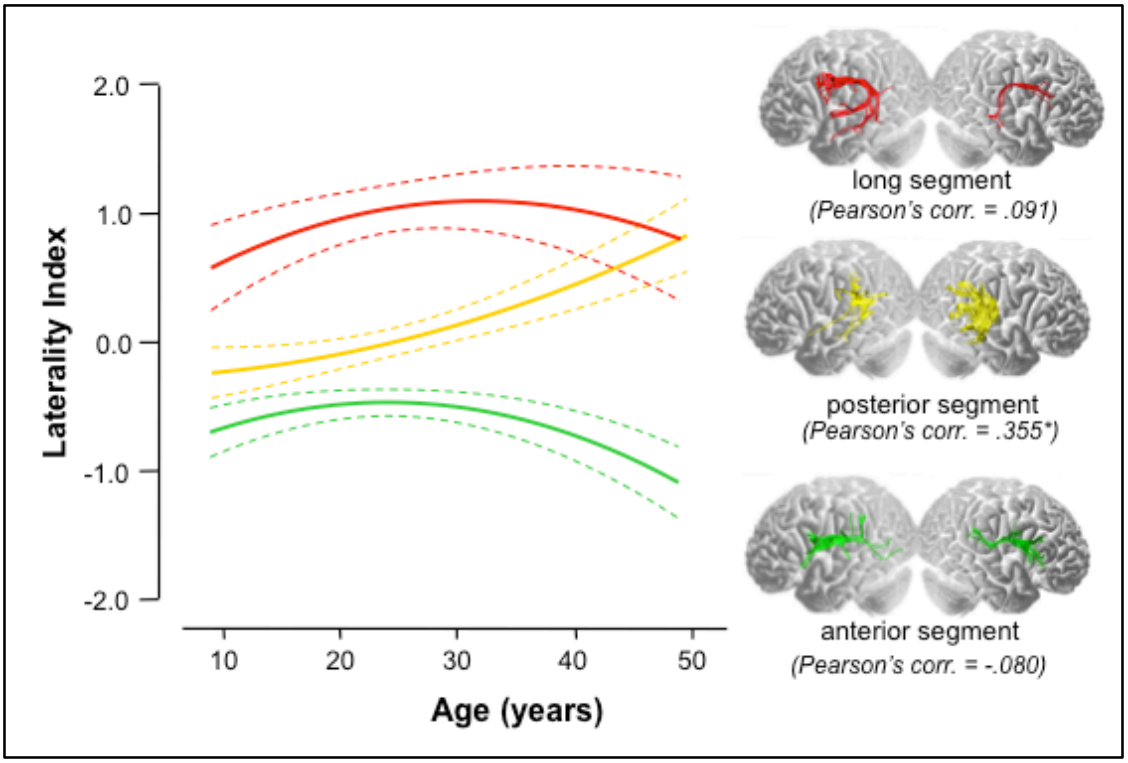

Fig 2.3.1. Age-related differences in the laterality index (number of streamlines) for the posterior, long and anterior segment of the arcuate bundle (quadratic fit lines ± mean 95% confidence intervals). Laterality index of 0.0 represents bilateral representation; 2.0 extreme left asymmetry; -2.0 extreme right asymmetry. The variance at extreme of age is wider due to the smaller number of subjects in that age range. *p< 0.001.

At the age of 9 there was a statistically significant difference in the laterality index of the three segments. The laterality index of the long direct segment showed positive values (.95 ± 1.11, age range 9-12 years) indicating a left lateralisation, whereas the indirect posterior (.04 ± .70, age range 9-12 years) showed no lateralisation and the anterior segments (-.56 ± .65, age range 9-12 years) indicated a right lateralisation. This suggests that the direct long segment has an asymmetrical distribution from an early age with greater volume in the left as compared to the right. A correlation analysis between laterality index (number of streamlines) and age (Table 2.3.1) revealed a statistically significant correlation for the posterior indirect

segment, whose values increase positively with age (Pearson's correlation = .355, p<.001), leading to a shift of lateralisation from bilateral to the left hemisphere. There was no significant correlation between age and the anterior indirect (Pearson's correlation = -.080, p=.53) and long direct segment (Pearson's correlation = .091, p=.34).

Table 2.3.1. Correlation between age and the lateralisation indices

Laterality Index	Age (all subjects)	Age (males)	Age (females)	M/F differences (Z-obs)
Anterior (SL)	-0.080	-0.109	0.018	-0.618
Long (SL)	-0.091	0.179	-0.073	1.20
Posterior (SL)	0.355*	0.439*	0.219	1.17
Anterior (FA)	0.044	0.080	-0.103	0.879
Long (FA)	0.065	-0.002	0.235	-1.13
Posterior (FA)	0.240	0.252	0.235	0.076
Anterior (MD)	-0.106	-0.087	-0.142	0.256
Long (MD)	-0.020	0.027	-0.143	0.808
Posterior (MD)	-0.212	-0.341	0.145	-2.37**

SL, streamlines; FA, fractional anisotropy; MD, mean diffusivity; *Pearson correlation is significant at p < 0.001; ** significant differences between genders

To understand whether this shift in lateralisation to the left of the posterior segment is associated with an increase in the number of streamlines in the left hemisphere or a decrease in the number of streamlines in the right hemisphere, a correlation analysis with the absolute values of the number of streamlines for each side was undertaken. This analysis shows a negative correlation between the number of streamlines in the right posterior segment (Pearson's correlation = -.366, p<.001) with age. There were no significant correlations with age for the number of streamlines in the left posterior segment, left and right anterior and left and right long segment (Table 2.3.2).

Table 2.3.2. Correlation between age and the number of streamlines

Segments (absolute values)	Age (all subjects)	Age (males)	Age (females)	M/F differences (Z-obs)
Anterior left (SL)	-0.070	-0.122	0.017	-0.645
Long left (SL)	0.107	0.117	0.104	0.051
Posterior left (SL)	-0.053	0.037	-0.112	0.689
Anterior right (SL)	0.078	0.097	-0.022	0.547
Long right (SL)	-0.087	-0.157	0.050	-0.962
Posterior right (SL)	-0.366*	-0.395*	-0.325	-0.385

SL, streamlines; *Pearson correlation is significant at $p < 0.001$

These results suggest that the lateralisation of the perisylvian language pathways is a dynamic process; and that for some of the tracts has already been completed before adolescence (e.g. long and anterior segments). In contrast, for the temporo-parietal connections (posterior segment) the lateralisation continues throughout adolescence and early adulthood. These differences occur in the right hemisphere and consist of a reduction in the number of streamlines.

In order to understand whether there is a critical period for these changes to occur, a *voxelwise analysis* was performed using visitation maps derived from the tractography dissections. For each segment a binary map was produced and averaged across subjects. A moving average window was applied to visualise age-related modifications in the volume of the specific segments. Compared to the number of streamlines this method represents an alternative estimate of the volumetric differences occurring during development. Figures 2.3.2-5 show the results of this analysis for each segment.

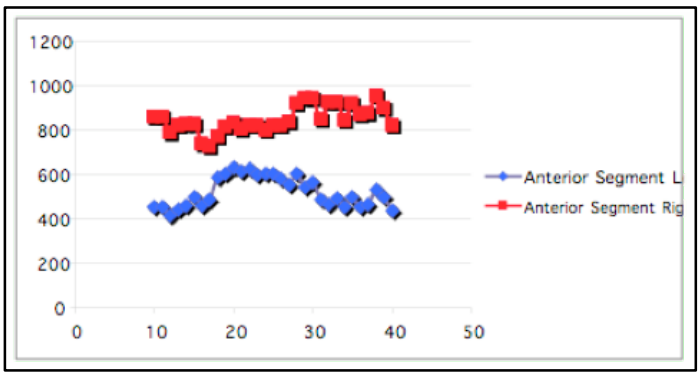

Fig 2.3.2. Age-related volumes of the anterior indirect segment in the right (in red) and left (in blue) hemisphere

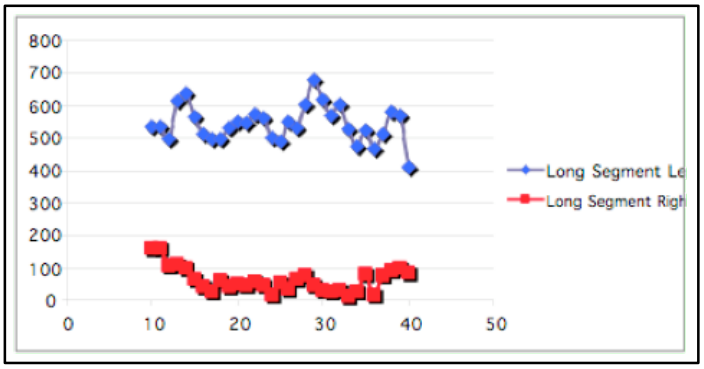

Fig 2.3.3. Age-related volumes of the long direct segment in the right (in red) and left (in blue) hemisphere

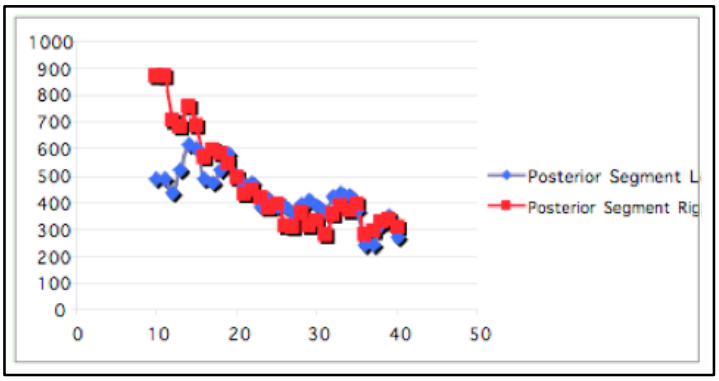

Fig 2.3.4. Age-related volumes of the posterior indirect segment in the right (in red) and left (in blue) hemisphere

As can be seen from the Fig 2.3.4, before adolescence there is a clear asymmetry in the volumes of the posterior segment, with greater values in the right hemisphere. By the age of 13 and throughout adolescence the volume of the right posterior segment decreases, whereas the volume of the left posterior remains almost unchanged. These changes continue also into early adulthood. The analysis confirms the early right lateralisation of the anterior segment and left lateralisation of the long segment and their minimal changes throughout adolescence (for an overview see Fig 2.3.5). These results suggest that adolescence is a critical period for brain development, during which posterior connections of the right hemisphere undergo intense reorganisation and lead to a change in lateralisation patterns.

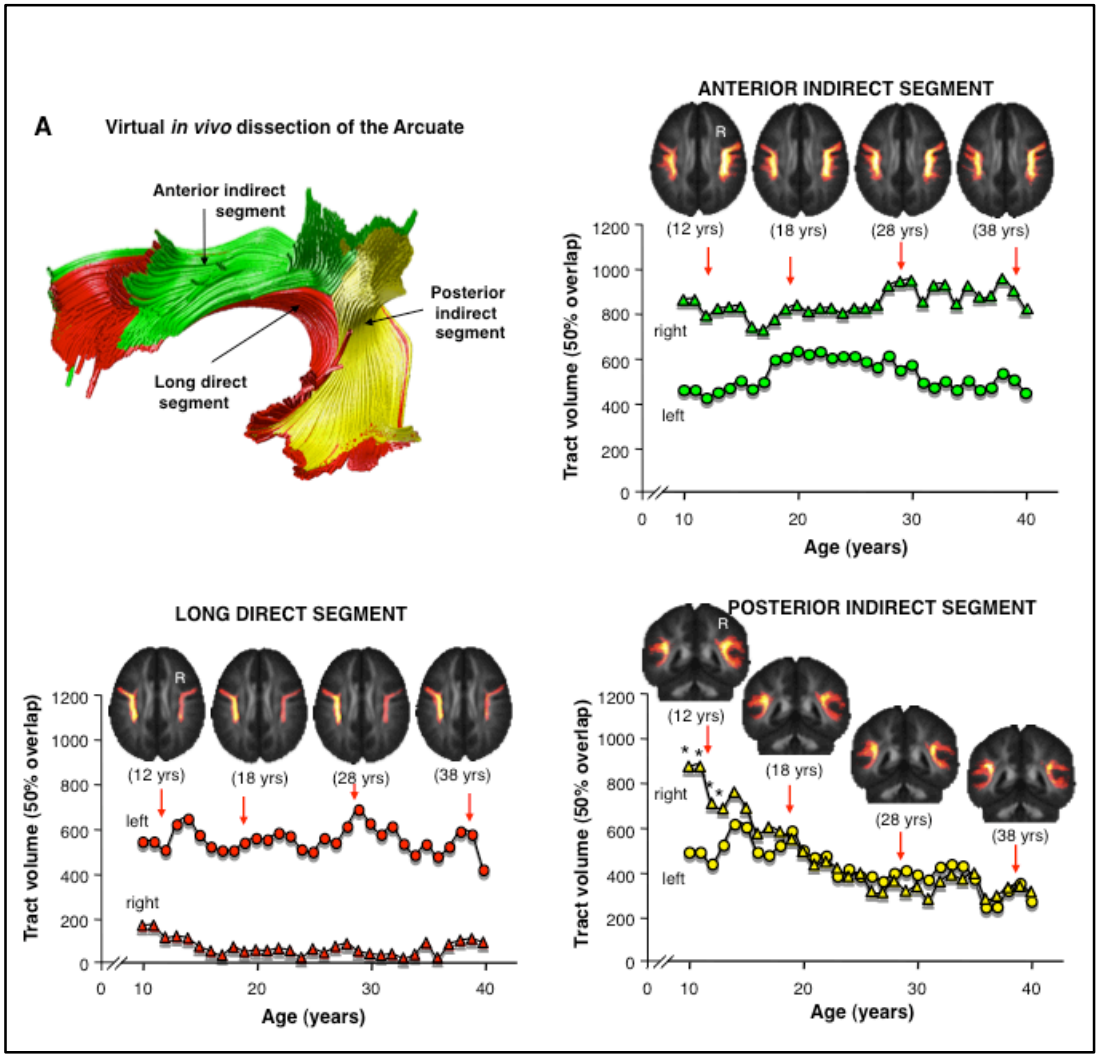

Fig 2.3.5. Visualisation of the age-related volumetric differences of the long, anterior, and posterior segments of the bilateral arcuate fasciculus. Tract volume with 50% overlap represents the volume of the tract that is common in at least 50% of the subjects.

Microstructural age-related differences in perisylvian language pathways

Microstructural differences in the arcuate bundle can be studied by sampling fractional anisotropy (FA) and mean diffusivity (MD) for each single tract. FA is a scalar measure that reflects the degree to which the diffusivity depends on the orientation in which it is measured, and is considered an index of microstructural order and integrity of fibres, whereas MD is a scalar measure of the total diffusion within a voxel. Correlation analysis of the absolute values of MD in the right and left hemisphere revealed a statistically significant negative correlation between age and mean diffusivity in bilateral posterior (Pearson's correlation = -.573, p< .001; Pearson's correlation = -.602, p< .001 respectively) and anterior segments (Pearson's correlation = -.562, p< .001; Pearson's correlation = -.595, p< .001 respectively), and long left segment (Pearson's correlation = -.540, p< .001) of the arcuate fasciculus (see Table 2.3.3). No significant correlation between absolute values of FA and age were observed.

Table 2.3.3. Correlation between age and FA, MD absolute values

Segments (absolute values)	Age (all subjects)	Age (males)	Age (females)	M/F differences (Z-obs)
Anterior left (FA)	0.072	0.193	-0.234	2.12**
Long left (FA)	0.035	0.145	-0.228	1.79
Posterior left (FA)	0.116	0.204	0.131	0.366
Anterior right (FA)	0.170	0.305	-0.133	2.14**
Long right (FA)	-0.080	0.078	-0.299	1.53
Posterior right (FA)	-0.118	-0.072	-0.060	-0.047
Anterior left (MD)	-0.595*	-0.650*	-0.382	-1.77
Long left (MD)	-0.540*	-0.627*	-0.178	2.62**
Posterior left (MD)	-0.602*	-0.669*	-0.330	-2.22**
Anterior right (MD)	-0.562*	-0.628*	-0.303	-2.026**
Long right (MD)	-0.173	-0.562*	-0.250	-1.48
Posterior right (MD)	-0.573*	-0.622*	-0.386	-1.51

SL, streamlines; FA, fractional anisotropy; MD, mean diffusivity; *Pearson correlation is significant at $p < 0.001$; ** significant differences between genders

Age-related differences between genders

To assess age-related gender differences in the perisylvian language pathways Z-obs (correlation curves) analysis was performed for microstructural differences (FA and MD), laterality index and the number of streamlines in each hemisphere. A significant gender difference was found using correlation analysis between age and the lateralisation index of MD of the posterior segment (males= -.341 compared to females= .145, Pearson's correlation = - 2.37, p< .001). This result suggests that different processes are occurring in the posterior indirect segment depending on the gender, with the lateralisation of MD increasing with age in females and decreasing in males (see Table 2.3.1). However, these individual correlations were not significant, and thus provide no further clue on the exact mechanisms that might be leading this gender difference.

The correlation analysis between age and FA and MD absolute values (Table 2.3.3) revealed a significant gender difference in FA and MD of the anterior right segment (Pearson's correlation = 2.14, p< .001; Pearson's correlation = -2.026, p< .001 respectively), and in the MD of the long left (Pearson's correlation = 2.62) and posterior left (Pearson's correlation = -2.22, p< .001) segment over time. Furthermore, Pearson's correlation between age and MD was statistically significant in all three perisylvian segments of both hemispheres in males - showing that MD decreases over time; but not in female participants, where only a trend of MD decrease with age was observed.

The analysis of microstructural changes (Table 2.3.4) showed a significant difference among genders in the absolute values of FA of the left posterior (males= .41± .03 compared to females= .43± .02, p< .001) and right posterior segment (males= .41± .02 compared to females= .43± .02, p< .001) of the arcuate fasciculus, with females exhibiting higher anisotropy values.

Table 2.3.4 FA and MD absolute values of the three segments of the arcuate fasciculus

Segments (absolute values)	Age (all subjects)	Age (males)	Age (females)	M/F differences (Z-obs)
Anterior left (FA)	.44 ± .03	.43 ± .03	.44 ± .02	.30
Long left (FA)	.47 ± .03	.47 ± .03	.48 ± .02	.13
Posterior left (FA)	.41 ± .02	.41 ± .03	.43 ± .02	< 0.001*
Anterior right (FA)	.45 ± .03	.45 ± .03	.46 ± .02	.20
Long right (FA)	.44 ± .10	.46 ± .03	.42 ± .15	.09
Posterior right (FA)	.42 ± .02	.41 ± .02	.43 ± .02	< 0.001*
Anterior left (MD)	.73 ± .03	.73 ± .04	.73 ± .02	.91
Long left (MD)	.73 ± .04	.73 ± .04	.72 ± .02	.81
Posterior left (MD)	.75 ± .03	.75 ± .04	.74 ± .04	.67
Anterior right (MD)	.72 ± .03	.72 ± .04	.72 ± .02	.76
Long right (MD)	.69 ± .14	.72 ± .04	.64 ± .22	.02
Posterior right (MD)	.75 ± .03	.75 ± .04*	.75 ± .02	.93

FA, fractional anisotropy; MD, mean diffusivity; *p values are significant after Bonferroni correction

No significant difference was observed for the absolute values of MD in the single tracts. In addition, no significant gender difference was observed in the laterality index of FA and MD values (Table 2.3.5), the number of streamlines in left and right hemisphere (Table 2.3.6), and in correlations between age and the number of streamlines (Table 2.3.2).

Table 2.3.5 Laterality indices of the three segments of the arcuate fasciculus

Laterality Index	All subjects	Males	Females	M/F differences (p-values)
Anterior (SL)	-.56 ± .65	.55 ± .73	-.59 ± .49	.78
Long (SL)	.95 ± 1.11	.82 ± 1.15	1.17 ± 1.01	.13
Posterior (SL)	.04 ± .70	.04 ± .66	.03 ± .78	.94
Anterior (FA)	-.04 ± .11	-.05 ± .13	-.04 ± .06	.57
Long (FA)	.11 ± .40	.03 ± .05	.23 ± .62	.04
Posterior (FA)	-.001 ± .06	-.007 ± .07	-.003 ± .04	.75
Anterior (MD)	-013 ± .02	.012 ± .02	.015 ± .025	.60
Long (MD)	.32 ± .48	.31 ± .49	.34 ± .47	.74
Posterior (MD)	.0004 ± .02	.0022 ± .02	-.0026 ± .018	.27

SL, streamlines; FA, fractional anisotropy; MD, mean diffusivity; *p-values significant at the < 0.001 level;

Table 2.3.6 The number of reconstructed pathways in both hemispheres of the three segments of the arcuate fasciculus

Segments (absolute values)	All subjects	Males	Females	M/F differences (p-values)
Anterior left (SL)	56.5 ± 34.4	59.3 ± 36.4	51.7 ± 30.6	.28
Long left (SL)	43.18 ± 28.7	42.7 ± 29.6	44 ± 27.6	.82
Posterior left (SL)	87.6 ± 58.2	78.7 ± 38.8	103.3 ± 80.2	.04
Anterior right (SL)	97.5 ± 44.5	99.8 ± 45.9	93.3 ± 42.2	.48
Long right (SL)	20.1 ± 26.4	23.4 ± 29.4	14.4 ± 19.4	.09
Posterior right (SL)	89.5 ± 60	70.7 ± 50	106.7 ± 72	.03

SL, streamlines

2.4 Discussion

The present study utilised diffusion tractography in healthy children and adults to investigate age-related differences in white matter anatomy of the perisylvian language pathways. This is the first time that a comprehensive account of the maturational trajectories of the three segments underlying the fronto-temporo-parietal language network has been reported, revealing different developmental models that ultimately affect the establishment of heterogeneous lateralisation patterns.

The main findings of this study are generally consistent with earlier post-mortem (Yakovlev and LeCours, 1967), MRI volumetry (Giedd, et al., 1999; Paus, et al., 1999; Sowell, et al., 2003) and DTI studies (Barnea-Goraly, et al., 2005; Schmithorst, et al., 2002) which reported continued white matter maturation throughout childhood and adolescence. This conclusion was derived from several lines of results that emerged. First, the lateralisation of the perisylvian language network, based on the number of streamlines and volume, differed between different segments already at our first observation at the age of 9. The long direct segment showed early left lateralisation with minimal changes during early adolescence and adulthood, while the indirect posterior segment exhibited a significant shift in lateralisation from bilateral to left as a function of age. This shift was due to a decrease in the number of streamlines of the posterior segment in the right hemisphere. The anterior indirect segment showed early right lateralisation with no significant difference occurring later on in life. Second, on the basis of an examination of the microstructural properties (fractional anisotropy (FA) and mean diffusivity (MD)) specific to the three segments of perisylvian network, significant differences were observed in the absolute values of MD that decreased over time in the posterior and anterior segments bilaterally and the left long segment, pointing to continuous maturational processes shaping the microstructure of these tracts. This significant decrease in MD over time was driven by sex differences, since in males MD decreased significantly with increasing age, while in females this decrease was observed only as a trend. MD was not the only measure that exhibited between-genders difference. The measures of FA also displayed significant gender effects in bilateral anterior segment. There was a trend towards an increase in the absolute values of FA with age in males, whereas a trend to a decrease in FA values with age was observed in females, with individual correlations failing to reach statistical significance.

Age-related differences in lateralisation patterns

Our results confirmed early left lateralisation of the long direct segment with minimal changes throughout adolescence and adulthood, consistent with the earlier reports (Barrick, et al., 2007; Dubois, et al., 2009; Nucifora, et al., 2005; Parker, et al., 2005; Powell, et al., 2006; Vernooij, et al., 2007). A lateralisation index based on the number of streamlines was also used by Lebel and Beaulieu (2009), who reached the same conclusion: that there was a consistent pattern of left asymmetry of the arcuate fasciculus (long segment) in children, adolescents and young adults, and this is stable across age and gender. Our findings provide further evidence that lateralisation of the long segment is constant from early adolescence to late adulthood.

The results of this study also suggest that different perisylvian pathways have different maturational patterns leading to different resulting asymmetries, consistent with the findings of Eluvathingal et al. (2007). However, in contrast with Eluvathingal et al's (2007) observation that long and posterior segments are likely to undergo substantial maturation by the age of 6 years, we showed that this is unlikely for the posterior segment that exhibited the most dynamic course of maturation. However, the difference lies in different measures used to observe maturation effects. Eluvathingal et al. (2007) used fractional anisotropy measures along the three tracts, while we analysed the volumetric measures to determine anatomical asymmetries. Our findings point to an early development of the long and anterior segment's asymmetries (left lateralisation for long and right lateralisation for anterior segment), while the development of posterior segment's asymmetry continues well into adolescence. At the age of 9 the posterior segment shows bilateral organisation, however by the age of 13 significant changes are observable. Around that age and throughout adolescence the volume of the right posterior segment decreases whereas the volume of the left segment remains unchanged, leading to the left lateralisation of this segment. This is in agreement with the findings of Paus et al. (1999) in whose study the variance of age related changes was lower in the left arcuate fasciculus compared to the right. If we consider a decrease in the number of streamlines and volume of the right posterior segment as an indirect measure of the loss or pruning of white matter, then our results support the hypothesis of Galaburda (1990) that lateralisation arises through axonal pruning and post-migrational cell loss, rather than an increase in neurons and axons. Importantly, a reduction in the number of streamlines of the right posterior segment was observed during early adulthood. Hence, our results support reports that adolescence is a critical period for brain development (Giedd, et al., 1999) - during which posterior temporo-parietal connections undergo intense reorganisation.

In line with our results, recent imaging findings suggest that the temporo-parietal cortical areas are the slowest regions to mature among the language network. For example Leroy et al. (2011) used an index based on the normalized T2-weighted magnetic resonance signal to quantify maturation within the linguistic network in 1- to 4-month-old infants. They found that the most immature structures of the linguistic network are not the inferior frontal cortices, as previously believed, but the superior temporal sulcus and the supramarginal gyrus, two structures connected by the temporo-parietal (posterior) segment. This is consistent with our results pointing that the posterior segment is a pathway that is the least mature within the perisylvian language network. It can, however, be argued that the differences Leroy et al. (2011) observed were not due to maturation but rather to differences in cellular organisation between the different types of cortices. However, the authors demonstrated that the signal difference between areas is not constant and that maturation, rather than cortical type, is the main cause of this difference between regions.

The dynamic pattern of lateralisation of the posterior segment observed in our study might be related to the increasingly left functional lateralisation during development. Thus, although previous structural studies observed a constant lateralisation pattern, which does not change from early childhood into adulthood (Lebel and Beaulieu, 2009), functional studies note an age-related increase in the degree of functional lateralisation favouring the left hemisphere during language processing tasks (Brauer and Friederici, 2007; Holland, et al., 2007; Perani, et al., 2011; Szaflarski, et al., 2006). Children's stronger reliance on the right hemisphere was

reflected in a more right functional lateralisation during language processing as compared to adults (Brauer and Friederici, 2007; Friederici, et al., 2010). Hence, it can be speculated that this increasing left functional lateralisation partly reflects the dynamic structural changes observed in the posterior indirect segment during development.

There are number of implications arising from the observation that the posterior segment has a different maturation trajectory compared to the long and anterior segments. Dynamic maturational pattern of the posterior indirect segment, that connects the temporal and parietal lobes, might be linked to its possible involvement in other functions besides language processing. It is known that the superior temporal sulcus hosts important functions besides language, such as social contact, audiovisual integration, and biological motion perception (Hein and Knight, 2008). Furthermore, the temporal and parietal lobes have been implicated in the "theory of mind" (ToM) - a model of the functional and anatomical basis of human ability to reason about other people, to predict and interpret their behaviour based on understanding of other's minds and thoughts. The relationship between language and ToM, which is the basis of social cognition, is still controversial. For example, Apperly et al. (2004) and Samson et al. (2004) found that patients with lesions affecting the left temporo-parietal junction (TPJ), the superior temporal cortex (STS), and inferior parietal (IP) regions were selectively impaired in ToM. Furthermore, a number of studies have reported increased responses in the TPJ during ToM tasks (Saxe and Kanwisher, 2003). These posterior regions seem critical for ToM reasoning, however the exact roles they have remains unclear. There is a debate whether TPJ is involved only in the preliminary stages of social cognition that "aid" ToM reasoning or ToM reasoning itself. Njomboro et al. (2008) gave one possibility that the left TRJ/STS/IP are responsible for higher-order mentalising processes that are required for belief reasoning, but not for decoding facial emotions. Perner et al. (2006) further found that the right TPJ was specialized for mental perspective tasks (false beliefs) while the left TPJ seemed to be associated with a broader range of tasks including mental states (false beliefs) as well as non-mental entities (false signs). It is a speculation whether the posterior indirect segment might be involved in any of the mentioned functions of the ToM model. However, it is possible that the posterior segment is implicated in additional functions besides language, and that it acts as a neural substrate for higher-mental processes like abstract thinking and reasoning, semantical processing etc.

Microstructural age-related differences

Many diffusion imaging studies reported microstructural age-related differences in the white matter of the human brain. Diffusion studies that examined developmental changes reported an increase in FA and a decrease in MD with increasing age (Barnea-Goraly, et al., 2005; Bonekamp et al., 2006; Dubois, et al., 2006; Neil, et al., 1998; Schmithorst, et al., 2002). Our study confirmed the latter observation in that the absolute values of MD decreased over time in the bilateral indirect segments (posterior and anterior) and left long direct segment. MD is the overall magnitude of water diffusion and is a sensitive indicator of maturational changes in brain tissue. A decrease in MD would reflect a decrease in brain water content and an increase in axonal membranes density (Neil, et al., 2002). However, unlike other DTI studies, there was no significant increase in the values of FA with increasing age. This is consistent with previous reports that

found MD measures to be more sensitive to age than FA measures (Barnea-Goraly, et al., 2005; Schmithorst, et al., 2002; Schneider, et al., 2004). In line with our results, a recent study also observed a significant age-related decrease in all three diffusivities that was not accompanied by significant increase in FA, in the left long segment and bilateral posterior segments of the arcuate fasciculus (Eluvathingal, et al., 2007). The authors interpreted these findings as continued changes in intrinsic characteristics of axons and/or changes in extracellular space, rather than progressive myelination. In our study we did not analyse the measures of parallel and perpendicular diffusivities, and therefore can only speculate on microstructural changes in developing brain focusing on two complementary diffusion indices FA and MD, and relying on the model assumptions made by Dubois et al. (2008, 2009). According to their model, a decrease in MD not followed by an increase in FA may be caused by proliferation and functional maturation of glial cell bodies and prolongations (oligodendro-glial cells and their processes, etc.) and intracellular compartments (neurofilaments, microtubules, etc.) indicative of a "pre-myelination" phase. However, we have to be aware that these changes are not due to true 'pre-myelination' since they are happening rather late in human development, from early adolescence to late adulthood. Therefore it is likely that they reflect a mixture of processes including continuous myelination and membrane proliferation – i.e. differences in the intrinsic characteristics of axons (that do not affect anisotropy measures, as reported by Beaulieu and Allen, 1994) or in extra-axonal/extracellular space. However, like every other scientific model, this one is also a simplification of possible biological explanations, and this should be taken into account. Furthermore, each voxel examined in a DTI dataset may contain a mixture of gray matter, white matter and cerebrospinal fluid. Therefore, a decrease in MD can potentially represent a change in any of these components, and thus may allow for several interpretations.

Gender differences

This study found significant age-related gender differences in the maturation of the perisylvian language network. Sex differences have previously been reported in the brain of the adult population by various DTI-MRI region-of-interest and voxel-based studies (Nucifora, et al., 2005; Peled, et al., 1998; Szeszko, et al., 2003) but a previous tractography study failed to observe them (Eluvathingal, et al., 2007). Sexually dimorphic developmental patterns were noticed in certain brain regions during childhood and adolescence (Giedd, et al., 1996). However, no study to date has specifically examined sex differences in the developmental patterns of the perisylvian language pathways.

In our study the genders differed significantly in the developmental patterns of the absolute values of MD in the dominant hemispheres (dominance measured by lateralisation index based on the number of streamlines): left hemisphere for the long and posterior segment, and right hemisphere for the anterior segment. It should be noted that in the male population all MD measures of the bilateral arcuate fasciculus showed a strong, statistically significant, negative correlation with age. Compared to males, females showed a trend towards a decrease in mean diffusivity in all the tracts, but this failed to reach conventional statistical significance. This difference was statistically significant between genders only in left long and left posterior segment, and right anterior segment. Therefore, males displayed a sharper, and more aggressive decrease

in MD compared to females, possibly indicating steeper maturational processes (e.g. membrane proliferation, intra-axonal and/or extra-axonal changes, etc.). However, when observing the absolute values of MD, there was a trend towards lower MD values in females as compared to males, so these differences might represent a 'catching-up' phase in the male population, rather than "faster" maturation.

The developmental patterns of FA measures also exhibited gender effects. The absolute values of FA in the bilateral anterior segment were positively correlated with age in males, but negatively correlated in females. However, these correlations again failed to reach statistical significance. If we use FA as an index of white matter maturation, then it can be speculated that the increasing FA with age in males, again indicate a faster, steeper and more aggressive maturation of the bilateral anterior segment, reflected in fibre coherence and alignment, axonal membrane integrity and myelination. However, noting that there is a trend towards higher anisotropy in females compared to males, these results might once more suggest a subtle 'catching-up' phase of the male population. Previously, a study by Schmithorst et al. (2008) observed that during childhood FA of the arcuate fasciculus increases in girls but decreases in boys. This finding provides justification for our observed maturational differences and the 'catching-up' phase noted in males during later stages of development (adolescence and adulthood). Further, this steeper white matter maturation during in males compared to females was also noticed for white matter growth during adolescence (Lenroot, et al., 2007) and consequent FA decline in adulthood (Rosenzweig, et al., 2012). This sexual dimorphism can be attributed to the rise of testosterone levels in males in adolescence (Perrin, et al., 2008, 2009), neuroprotective properties of oestrogen in females (Kochunov, et al., 2012), differences in sex chromosomes (Temple and Shephard, 2012), and other biological mechanisms (e.g. epigenetic factors).

Limitations and conclusions

DT-MRI is the only technique that allows us to identify large white matter pathways in the living human brain. It was therefore used to investigate the association between macro- and micro- structural properties and age. However the tensor model is not without limitations, and these have to be taken into account. DT-MRI tractography offers, at best, only indirect indices of tissue properties and a degree of uncertainty in tractography measurements (e.g. number of streamlines, FA etc.) always exists (Catani, 2007). A deterministic fibre tracking algorithm was used with tract-specific measurements (TSM) method to extract anisotropy and diffusivity values at regular intervals along the reconstructed fibres. Hence, tract-specific measurements are used to assess micro-structural differences within a particular tract. The TSM approach can suffer from a number of problems, such as operator-dependent placement of the seed regions from which the tracking is started. However, despite this operator dependence, it has been shown that anatomically faithful reconstructions of white matter fasciculi can be reproduced (Catani, et al., 2002). In addition, there are difficulties in resolving the crossing, 'kissing', and touching of different fibres, since in the regions where fibres cross, branch or twist the tensor model does not perform as well (artificially low FA values could be obtained) and artifactual reconstructions of the pathways (false negatives and false positives) are likely to occur (Basser, et al., 2000). However, all the tracts were visually inspected to confirm their anatomical correctness. Another limitation involves the spatial normalisation I used for visitation maps.

This is not as robust in children as it is in adult brains, because children's brains are smaller and thus due to the low spatial resolution it is possible that some partial-volume overlap between white matter and adjacent structures could more easily occur. Furthermore, the normalisation technique has its limitations in the fact that considerable variability exists among subjects, and so some individual differences (e.g. ventricular variances) were observable even after the normalisation was done. Although not a limitation *per se*, it is important to emphasize that my study was cross sectional, where data was obtained from different subjects of different ages, rather than a longitudinal study of the same subjects as they aged over time, which is the ideal method when investigating developmental patterns. Future extensions of this study should include larger male and female populations with a wider age range, or ideally longitudinal type of study. Furthermore, additional statistical modelling of the interplay between microstructural DTI metrics and the accompanying cognitive and behavioural changes with development would be useful. In the future, a combination of DT-MRI tractography with other MRI techniques will allow the information about the engagement of specific tracts during cognitive tasks to be extracted together with the information about the metabolic composition of the dissected pathways (Catani, 2007).

The study shows observable age-related differences in the maturation of the posterior indirect segment compared to the long and anterior segment of the arcuate bundle. It raises further questions regarding the posterior segment as being functionally distinct from the other two, and involved in more than just language processing. The ability to process and use language for communication involves both language and social cognition. In addition, language itself can be viewed as a set of processes. The likely role of the posterior indirect segment in higher mental processes gives good ground for further research into the relationship between language and social cognition. In-vivo quantification of white matter characteristics within specific perisylvian language tracts during development provides the basis for further research into the normal language neurodevelopment as an important step towards unravelling the reasons behind language deficits and pathological conditions implicated in many disorders (e.g. autism). In conclusion, our data suggest that the maturation of the perisylvian network is not a uniform process for all of its three segments, but instead differs and shows distinct developmental patterns throughout human lifespan. Lateralisation exhibits a dynamic pattern that for some segments finishes early in life (long direct and anterior indirect segment) while for the others (posterior indirect segment) continues well into adolescence. The study confirms the early left lateralisation of the long segment. The anterior segment shows an early right lateralisation, whereas posterior segment shifts from being bilateral to becoming left lateral, due to a decrease in the number of streamlines in the right hemisphere. Early structural asymmetries of the long and anterior segments of the arcuate fasciculus suggest that macro- and microscopic structural organisation and maturation of this network might have underlied the setting up for brain functional lateralisation. Significant negative correlation between age and the absolute values of the mean diffusivity indicate constant maturation with age diffused throughout the arcuate bundle. Finally, there were significant gender differences observed in the maturation of the perisylvian language microstructure, with males showing steeper maturation of certain perisylvian language tracts that might be due to a 'catching-up' phase; leading to the conclusion that language-related neurodevelopment differs among genders.

Chapter 3

Imaging genetics and twin methodology

3.1 Introduction

Quantitative genetics has been waiting for centuries to merge with the modern, up-and-coming, neuroimaging methodology. Once found, the new duo quickly became an influential field of study, often referred to as simply 'imaging genetics' - threatening to be the most promising technique for understanding genetic basis for variation in brain structure and function. Although genetic variation is what usually captures scientific curiosity, it is not *solus ipse* as the cause for variation. One of the most consequential findings that has emerged from quantitative genetic studies is the importance of various environmental factors in shaping individual differences of brain structure and function. This section will introduce the reader to the imaging genetics, and deal with the fundamental methodological concepts that made my research on language heritability possible, with a special focus on classical twin study design used in this PhD project (Chapter 4).

3.2 Historical context of quantitative genetic approach

The idea that a hereditary component is part of our ephemeral living makeup has been with us for centuries. The first known questions about the causes of human differences and similarities were sparked by the twins enigma. It was in 426 A.D. that Augustine of Hippo in Book V of the 'City of God' argued that highly discrepant life histories of the twins point to the failure of astrology and planetary influence on human destiny (Neale and Maes, 2002). Ancient Greeks attributed similarities of twins to their shared environment (maternal environment more precisely). Nevertheless, it was not until the pioneering work of Francis Galton in the nineteenth century that the study of twins received its deserved attention. Francis Galton (1875) was the first to systematically examine the effects of nature versus nurture on human behaviour although it is uncertain whether he knew of the distinction between monozygotic (MZ) and dizygotic (DZ) twins. He did realize that twin and family studies are the key experiments of nature upon which he could infer his observations about genetic and environmental interplay. He introduced the concept of a correlation coefficient as a measure of association between variables, which became the basis for future developments in twin methodology and quantitative genetics (Neale and Maes, 2002).

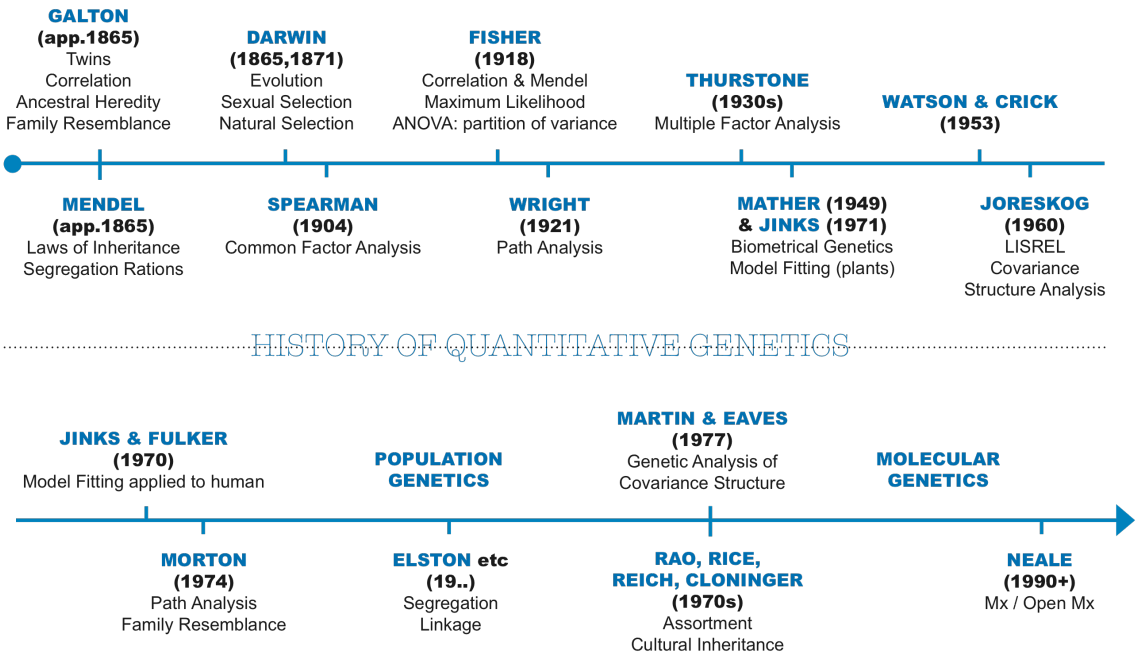

Fig 3.2 Graphical summary of the main streams of intellectual thought which converged to yield the ideas and methods that we use today in imaging genetics, which Neale and Maes (2002) discuss in their seminal book 'Methodology for Genetic Studies of Twins and Families'. The picture is not intended to be a comprehensive history of statistical or quantitative genetics, so a number of people whose work is extremely important to this discipline might be unaccounted for.

The systematic analysis of similarity between MZ and DZ twins was introduced by Siemens H.W. in 1924, who formulated the twin rule of pathology: any heritable disease will be more concordant in identical twins than in non-identical twins, and concordance will be even lower in non-twin siblings (Boomsma et al, 2002). Quantitative genetics has, since the nineteenth century and with the advances in statistics, blossomed into different areas of research, and has become an intrinsic part of behavioural, population, molecular, and imaging genetics. A number of thinkers contributed and made today's progress in genetic analysis possible. Some of them are shown in Fig 3.2 together with brief information regarding their main findings and ideas (for more on the history of quantitative genetics see Neale and Maes, 2002 Chapter 1, or Boomsma et al, 2002).

3.3 Twin methodology and classical twin study design

Today we are aware that most types of behaviour and traits (normal variations such as receptive and expressive language skills, or fractional anisotropy of the arcuate fasciculus) result from a complex interplay between environmental factors and multiple genes - thus named the polygenic model (Tandon and McGuffin, 2002). Individual differences in traits (phenotypes) of a population are studied as a total of genetic and environmental effects by means of twin and family methods. When behaviour genetic techniques are combined with neuroimaging studies the answers to how important the genes and environment are on the structure and function of the brain can be addressed. However, the information we obtain from imaging genetics is limited. It does not currently tell us the number of genes affecting the trait, the direction of these gene effects, or the specific identity of the genes exerting this influence (Medland and Hatemi, 2009).

The most common method used in quantitative genetics for initial exploration is a classical twin design which focuses on the variance rather than the means, and which I applied in this PhD study of the heritability of language pathways. The objective of the classical twin design is to examine the extent to which genetic and environmental factors influence variation around a population mean (Neale and Cardon, 1992). It is important to remember that the causes of variation that emerge relate to a particular population of genotypes at a specific time and place in their evolutionary and cultural history. Outcomes of the studies can be affected by factors that change the gene frequencies, the expression of genes, or the frequencies of different kinds of environmental influences. It is vital to be aware that results obtained relate to the causes of human differences, and may have almost nothing to do with the processes that account for the development of the mean expression of a trait in a particular population (Neale and Cardon, 1992). Hence, it is important to avoid expressions such as “FA in the arcuate fasciculus is genetic" when we really mean” individual differences in FA in the arcuate fasciculus are mainly genetic."

Two main types of twin studies exist: those based on twin pairs ascertained through affected probands and those based on population twin registers. The former is appropriate for investigating heritability of diseases, whereas the latter is better suited for studying common traits in the population (Rijsdijk and Sham, 2002). Both studies are based on the fact that there are two types of twins: monozygotic (MZ) that are genetically identical, and dizygotic (DZ) that share, on average, 50% of their genetic makeup. By comparing the observed correlations or concordances between them we can relate the studied traits of twins to their underlying genotypes and environments.

Recently it became possible to write structural equations in biometrical genetic theory, relating observed traits of twins to their underlying genotypes and environments. As a rule, a polygenic model is used in which the observed trait is influenced by several different loci on one or more chromosomes. This leads to the sources of genetic and environmental variation considered in behavioural genetics to be divided into:

- *additive genetic influence*, **A**, representing the sum of the effects of alleles at all loci that influence the trait;
- *non-additive genetic influences*, concerning the interactions between alleles at the same locus (dominance, **D**) or on different loci (epistasis);
- *common environmental factors*, **C**, shared by family members/twins, e.g. linguistic input children receive from the parents, socioeconomic status, rearing, childhood diet etc. In twin studies, shared environment is expected to contribute to the correlation of both MZ and DZ twins as long as they are reared together;
- *unique environmental factors*, **E**, specific to each individual e.g. illnesses, accidents, differential parental treatment, differential prenatal exposure, which results in differences among the twins. Importantly, E also includes the measurement error.

Geneticists distinguish between broad-sense heritability and narrow-sense heritability. Broad-sense heritability refers to the variance accounted for by all genetic factors (A+D), including the influence of gene dominance, epistasis and interactions between genes and environment. Narrow-sense heritability is the variance accounted for by additive genetic factors (A) alone, and represents the amount of genetic influence that is likely to be passed on to offspring (Stromswold, 2006). Narrow-sense heritability is studied in this PhD thesis.

The total phenotypic variance of a trait (**P**) is the sum of all these genetic and environmental effects, and can be expressed through equation:

$$P = A + D + C + E$$

Because of the difference in genetic proximity between twins with different zygosities, a classical twin design is able to yield these variance components. To elaborate this better, it suffices to know that MZ correlate 1 for additive genetic effects (A) (or narrow heritability) because they are genetically identical, whereas DZ twins correlate only half of that (0.5) because they share only half of their genes (Plomin, 2001). Conversely, both types of twins correlate 1 for environmental effects that both twins share (C), whereas unique environmental effects (E) that twins do not share is uncorrelated for both types of twins (for visual representation refer to Fig 3.3.1). As a consequence of these different degrees of correlations, a higher correlation in MZ compared to DZ twins would signify the higher proportion of genes shared among MZ twins. Similarly, the first impression of the importance of unique environmental factors can be obtained from the extent to which MZ twins do not resemble each other. On the other hand, if there is a high degree of similarity between MZ twins, as well as DZ twins, this can be interpreted as due to high shared environmental effects on the trait. A/C/E influences on the phenotype are given by parameters a, c and e, which are equivalent to the standardised regression coefficients of the phenotype observed on their respective latent (unmeasured) factors A, C and E. The amount of variance due to each source is the square of these parameters.

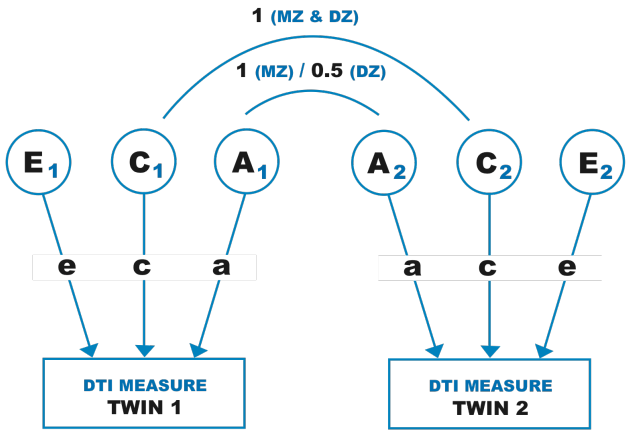

Fig 3.3.1 Path diagram of the univariate genetic ACE model used in this PhD study. The sources of phenotypic variation considered in this example are A, the additive genetic factors; C, the environmental influences shared by the twin pair and E, specific environmental factors that are unique to each twin member. a, c and e are path coefficients representing the relative contributions of A, C and E, respectively. Correlations between A_1 and A_2 is 1 for MZ twins that share all of their genes, and 0.5 for DZ twins that share only half of their genes. The correlation between C_1 and C_2 is 1 when the twins are reared together (and 0 when not). No interaction is assumed between the genetic and environmental factors within an individual.

It might be noticed from the Fig 3.3.1 that the effects of shared environment (C) and dominance genetic effects (D) are not included simultaneously in the model. This is because they are confounded in twin studies, and therefore cannot be tested together. The twin correlation pattern reveals which of the two effects is more likely. When the DZ twin correlation is less than half of the MZ correlation, dominance genetic influences are more likely. A common environment tends to make the DZ twin correlations greater than half the MZ correlations (Boomsma et al, 2002). DZ correlations of about half the MZ correlations suggest additive genetic influences but are also consistent with the presence of both C and D. Data on twins reared together do not contain enough information to determine both factors. However, if data on adopted twins are included (which can give an independent estimate of C) we can estimate the effects of both components (Rijsdijk and Sham, 2002). It is important to note that even twins separated at birth share the same pre-natal environment, so comparison of twins reared together and apart is only able to provide a simple test of the post-natal shared environment which should be taken into account when explaining the results (Neale and Maes, 2002).

Assumptions of the classical twin design

A number of assumptions are made in the classical twin study design (see Fig 3.3.2). If all four assumptions are met then classical twin study design can be performed. It is important to be aware of the implications of such assumptions and of the extent to which they are realistic in relation to the trait in question (Rijsdijk and Sham, 2002). The assumptions include the following:

- Gene-environment correlations and interactions are minimal for the trait;
- MZ and DZ twin pairs share their environments to the same extent;
- Mating in the population occurs at random;
- Twins are not different from the general population in terms of the trait.

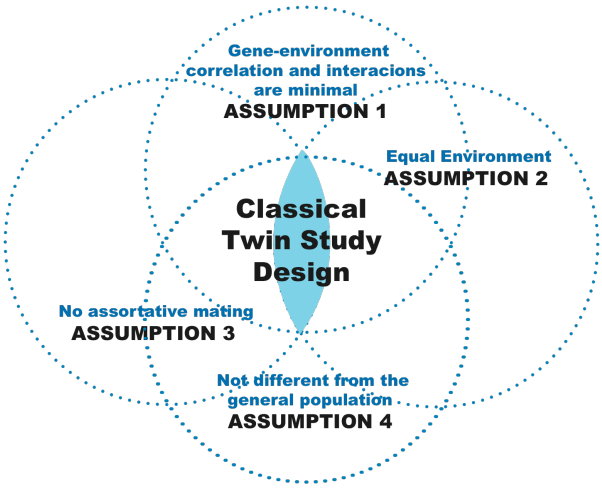

Fig 3.3.2 Assumptions of the classical twin study design

The assumption that has received most criticism in the classical twin study design is the assumption of equal environment (Phillips, 1993), which states that trait-relevant environments of MZ twin pairs are not more correlated than that of DZ pairs. This allows the twin model to calculate the genetic influences based on the extent to which MZ twins are more alike than DZ twins. Criticism dwells around the idea that shared environment might be more alike for MZ twins because they might experience more similar environments while growing up (e.g. the same dressing, sharing friends). However, various checks, such as incorporating environmental measures in twin studies and examining the effects of mistaken zygosity, suggest that the equal environmental assumption is generally valid (Plomin, 2001).

Falconer's formula of heritability

Early twins studies used Falconer (1960) transformations to estimate proportion of variance due to additive genetic (A), nonadditive genetic effects (D), common environment (C) and unique environment (E) effects from the MZ and DZ correlations. Falconer heritability (h^2) is a rough estimate of the relative contribution of additive genetic effects to the total phenotypic variance, obtained by doubling the difference between MZ and DZ twin correlations. All the variance estimates are based on twin correlations where *r* is an intra-class correlation coefficient:

$h^2 = 2(r_{MZ} - r_{DZ})$ assuming $D \cong 0$;

$C = 2r_{DZ} - r_{MZ}$

$D = 2r_{MZ} - 4r_{DZ}$

$E = 1r_{MZ}$

The intra-class correlation coefficient (ICC) is a statistical measure for the strength and direction of resemblance between two variables (or two family members). It can vary between -1 (no resemblance) and +1 (identical). ICC refers to the correlation in defined subgroups, such as in MZ or DZ pairs (Boomsma et al, 2002). This approach is not adequate for testing explicit models for individual differences and ignores information available in variances and covariances important for analysing sex and generation differences. In the past years this method was replaced by more advanced analyses techniques in which genetic covariance structure models are employed by special purpose software, where data can be analysed by means of maximum likelihood (ML) techniques. Packages used for this modelling are LISREL (Joreskog and Sorbom, 1986) and Mx/OpenMx (Neale, 1999).

3.4 Introduction to Structural Equation Modelling approach for analysis of twin data

Structural equation modelling (SEM), also known as covariance modelling, is a more advanced method, which in contrast to the Falconer analysis is capable of explicitly testing how genetic and environmental factors contribute to explaining individual differences by fitting a genetic model to the observed data and testing the model fit. By genetic model we mean a formal, mathematical statement which mediates between the logic of the theory and the reality of the data (Neale and Maes, 2002). This method estimates regression coefficients ('parameters') between latent (unmeasured, e.g. A) and measured (e.g. mean diffusivity) variables. This is done by using numeric optimisation of a likelihood function and producing parameter values that provide the best fit to the data. The output then informs modification of the parameters and the process is repeated until the likelihood of the observed data is maximised, that is, the model best approximates the data (Neale and Cardon, 1992). The difference between the covariance structure of the observed data and that predicted by the genetic model is thus minimal.

Advantages of the SEM approach are that assumptions can be made explicitly and can be tested, that parameters can be estimated with their standard errors or confidence intervals, and that the programs provide a chi-square test of the goodness-of-fit of the tested genetic model. In genetic model fitting a series of structural equations are solved, which allow comparison of alternative models in order to estimate genetic and environmental parameters that best fit the observed twin co-variations (Boomsma et al, 2002). Parameters A or C, or both, can be removed from the univariate ACE model to generate sub-models (i.e. AE, CE, E) that can be tested via likelihood ratio tests. Further, in genetic model fitting more than two groups of twins can be analysed simultaneously, and sex differences in parameter estimates and significance of parameters tested.

Also, the univariate analyses (one variable tested - see Fig 3.3.1) can be extended to multivariate (multiple variables) designs (Neale and Cardon, 1992). Finally, SEM is appropriate for both human and animal quantitative genetic data (Rijsdijk and Sham, 2002).

Modelling twin data in OpenMx

Many SEM programs are available on the market, but the package OpenMx (University of Virginia, Virginia, USA) was specifically developed to model genetically sensitive data in a flexible way, and represents an advancement over previous package Mx (Neale, 1999). For this PhD study we have used OpenMx to analyse the twin data. The advantages of OpenMx lie in the fact that data handling and data manipulation is flexible. Data can be entered either as summary statistics (e.g. covariance matrices and mean vectors) or raw data. Raw data allow greater flexibility: missing data problems are handled automatically; it is possible to fit finite mixture distributions and it is easy to specify continuous moderator variables. In addition, OpenMx allows for testing both dichotomous moderator effects (e.g. sex) and continuous moderator variables (e.g. age) (Rijsdijk and Sham, 2002).

3.5 Limitations of imaging genetics

Are the tools of classical twin design limited when applied to complex human traits (e.g. language and 'language' anatomy)? Understanding biometrical properties of human complex traits in neuroimaging, such as the anatomy of language in the brain, can prove challenging for a number of reasons.

Several standard limitations exist in the quantitative genetics methodology that can affect the results of imaging genetics: assumptions of twin design not met, ascertainment bias (systematic distortion in measuring the true frequency of a phenomenon - trait or disease), problems with phenotypic assessment, lack of follow-up of the phenotypes over time and environmental noise that can arise, for example, from developmental variation (Boomsma et al, 2002).

Besides these, it needs to be established whether results of the twin studies are applicable to non-twin populations. The results might be regarded applicable only to the extent that twin and singleton brains are alike. A reason to suspect differences is that as a group, twins are more likely than singletons to experience adverse prenatal (before birth) and perinatal (during or immediately after childbirth) events that may affect brain development. However, Ordaz and colleagues (2010) found no significant differences in the brain structures of twins compared to singletons in healthy paediatric data, neither did Hulshoff Pol et al (2002) in adult data. However, further studies are needed to include different imaging methods and different demographic samples in order to infer any sensible generalisation of results to non-twin populations.

Results of power studies show that at least 200 pairs are needed for obtaining a reasonable estimate of the degree of genetic influence on a highly heritable trait. For intermediate or low heritable traits, 10–20 times these numbers are required. The same is true for detecting shared environmental effects and non-additive genetic effects (Rijsdijk and Sham, 2002). This proves to be especially challenging for neuroimaging studies, where the sample size is significantly smaller compared to, for example, behavioural studies. There is a lack of consensus regarding formal power calculations in MRI and DTI studies, but usually their group sizes are too small for obtaining any viable quantitative genetic results, compared to thousands in population study samples. Lack of sufficient power in neuroimaging studies necessary to detect A and C can be helped by reporting C and A in combination (if they are statistically significant together), and expressing them as 'familial effects'. Classical twin design is further restrained by being unable to discern between C and dominant genetic effects (D). It does not mean that both effects are not present, and it is a limitation affecting the interpretation of results. Additionally, the areas in which non-genetic factors are the chief contributors to variance are extensive. However, in twin studies it is not possible to separate various sources of shared or unique environmental effects. For example, unique environmental influences will always include the measurement error thus making interpretation more difficult.

Using the advantages of the twin methods discussed here, the next Chapter will review the genetics of the brain and language, and introduce the second diffusion tractography study of this PhD project that examined the heritability of the perisylvian language pathways in the healthy human brain.

Chapter 4

Heritability of the perisylvian language pathways - linking genes, brain and language

4.1 Introduction and general aims

We have observed that in typical development the perisylvian language network undergoes different maturational trajectories leading to differences in anatomical make up (see Chapter 2). How experience impacts on the anatomy of these pathways when compared to genetic mechanisms is not known, yet the degree to which genes and environment determine brain structure is of fundamental importance. There must be many ways in which genetic and non-genetic influences combine to determine the differences in the anatomy of the perisylvian language pathways. The task of this chapter is to try to discern how genetic factors affect the neural bases of language in adulthood.

An exploratory study was performed investigating 86 genetically identical and fraternal male twins using diffusion tensor imaging (DTI) tractography and quantitative genetics approaches to estimate the relative contribution of genes and environment to perisylvian white matter anatomy, and to explore the connections between genetics of perisylvian white matter and maturation. This was a retrospective study that used already acquired data from several projects over the last five years, with demographic information limited to age, gender and handedness. An account of the genetic mechanisms that control the variability of different aspects of perisylvian white matter is crucial for understanding normal and pathological language function.

In order to better understand the sculpting of brain language connections, this study tested the hypotheses that (i) there are differences in the heritability patterns among distinct perisylvian language pathways (ii) heritability of the lateralisation patterns varies between those tracts that lateralise early (long and anterior segment) as compared to those that have a more dynamic lateralisation pattern (posterior segment).

4.1.1 Heritability of variation in brain structure and function

In neuroscience we are faced with the inevitable question of what caused our brains to be of a certain size and shape. Influences of nature and nurture on the brain are not independent, since genes function through the environment, especially if they involve susceptibilities to environmental stressors (Thompson, et al., 2001). We are aware that complex interplay is at place, between genes, genes and environment, and other factors (e.g. hormonal) - producing the end product, which is our brain. But how do we know to what extent each of these factors plays a role in shaping our brain? It was not until the recent development of brain imaging methods and quantitative genetics that the answer to this question became available (for a review see Peper, et al., 2007). Applying these two methods in parallel we can measure the degree of genetic control over variability in brain structure and function. This means that we can get an idea of the roles that both genes and environment play, though we cannot locate specific genes or understand the molecular pathways involved. However, we can inform future genetic linkage and association studies where to direct their research focus in their hunt for genes.

The traditional and most common method used in the quantitative genetics of neuroimaging is a classical twin design that compares monozygotic (MZ) and dizygotic (DZ) twin pairs (see Chapter 3). Although it might seem unnecessary to consider brain morphological and volumetric differences between MZ twins because of their identical genotype, there are various reasons why MZ twins might still have different brain anatomical features (see Chapter 3.5). Until 2007, there were approximately 75 twin reports using magnetic resonance imaging (MRI) (Schmitt, et al., 2007). Although there were some studies using functional MRI, the vast majority of twin studies have focused on anatomy. Of these, almost half, 35 reports, focused on normal brain structure. Half of these 35 reports have used the SEM technique, with the other half basing their estimates on Falconer analysis. The reason why anatomical MRI is used more often in twin designs most likely lies in the fact that it allows for several options in image processing and the wider/earlier availability of more conventional MRI technology for structural assessment. Structural MRI yields both volumetric (measurement of volumes) and form analyses (measurement of shape) at multiple levels of spatial resolution (Schmitt, et al., 2007). This section will review recent anatomical and functional twin studies using MRI, and discuss how heritable the variations of normal brain structure and function are, with the focus on the aetiology of variations in brain structure.

4.1.1.1 Heritability of brain phenotypes

Global brain volumes

A large body of literature has revealed that the most heritable neuroanatomical feature in twins is global brain volume. Recent imaging studies have shown that genetic factors account for 64-97% of total cerebral volume across the lifespan, ranging from young children (Eckert et al, 2002; Peper, et al., 2009; Wallace, et al., 2006) and adolescents (Pennintgon, et al., 2000) to adults (Baare, 2001; Bartley, Jones and Weinberger, 1997; Brun, et al., 2009; Posthuma, et al., 2002; Tramo, et al., 1998; Wright, 2002) and elderly population (Carmelli, et al., 1998; Geschwind, et al., 2002). This finding was supported by a recent review of 17 anatomical twin studies applying volumetric MRI, voxel-based morphometry (VBM), and DTI analysis, consistently noting the high heritability of global brain volume (Peper, et al., 2007).

When investigating the genetic basis of global brain volumes, we need to be aware that brain maturation is an ongoing, dynamic process (see Chapter 2.1.1). As a consequence of continuing changes, global gray matter volume initially increases, but then decreases around puberty (Giedd, et al., 1999; Gogtay, et al., 2004; Sowell et al., 2002, 2004; Thompson, et al., 2000) while global white matter volume increases linearly over time (Barnea-Goraly, et al., 2005; Giedd, et al., 1999; Paus, et al., 1999). However, regardless of these maturational changes, the heritability of global grey and white matter volume is consistently high throughout life. The first twin-sibling study to measure the genetic contributions to variation in global grey and white matter found heritability of 82% for grey and 88% for white matter volume in adults (Baare, 2001). Later studies confirmed that genetic factors account for at least two thirds of the phenotypic variance in gray and white matter volume (for a review see Schmitt, et al., 2007).

What are the possible explanations behind these findings? High heritability of gray matter volume may imply that inter-individual variation in cell-body volume is largely driven by genes, and only marginally modified by experience. A similar scenario can be observed for white matter. However, it should be remembered that genes and environment are not independent of each other, and that genetic factors can drive the exposure to certain environmental settings and relevant experiences.

Regional brain differences

Heritability estimates for neuroanatomical substructures are less known and the role of genetics less clear than for global volumetric measures. This is mostly due to two factors: a dearth of research, which results in one or two estimates of heritability reported for a given region, and frequent disagreements between studies (Schmitt, et al., 2007). Nevertheless, research efforts have indicated that genetic effects vary regionally within the brain. Overall, cortical regions involved in language, executive function, and emotional regulation appear to be more heritable than other areas (for a review see Peper, et al., 2007, and Schmitt et al, 2007).

In order to investigate heritability of regional grey matter, most studies tend to parcellate cerebral gray matter into cortical regions corresponding to Brodmann's areas (Wright, et al., 2002) or use point-wise (not a region-wise) analysis of cortical measures (Joshi, et al., 2011). So far, the most consistent finding is high genetic control of the frontal grey matter. The grey matter areas of the brain involved in language, process and rule learning are under tighter genetic control in terms of anatomy than other areas of the brain like the temporal and parietal (Thompson, et al., 2001; Toga, et al., 2006). This is not to say, however, that other brain regions are not under strong genetic control. Overall, high heritability estimates were revealed for regional amounts of grey matter in medial frontal cortex, Wernicke's area, Heschl's gyrus and postcentral gyrus, while moderate to high heritabilities were observed for densities in Broca's area, anterior cingulate, hippocampus, amygdala, grey matter of the parahippocampal gyrus (Brouwer, et al., 2010; Peper et al, 2007, 2009; Thompson, 2001).

There also seems to be a significant interaction between genetic control of grey matter and age. Findings suggest that heritabilities of regional grey matter densities as well as cortical thickness might increase with age. Moderate influences of genetic factors on cortical thickness have been found in children and adolescents, mainly in the frontal regions (Lenroot et al., 2007; 2009; Schmitt, et al., 2008). However, in adults, the heritability estimates for some gray matter areas (Thompson, et al., 2001; Wright, et al., 2002; Hulshoff Pol, et al., 2006) are more pronounced than in children. Other authors, like Wallace, et al. (2006) in contrast observed a reduction in heritability of grey matter volumes with increasing age, while white matter volume heritability increased with greater age - perhaps providing the control mechanism of continuous increase of white matter volume during development (Paus, et al., 1999).

Regarding the heritability of localised white matter, a similar picture can be observed, with genetic effects varying regionally within the brain. Currently, genetic analyses are being done mostly through analysis of white matter integrity using diffusion measures (Chiang, et al., 2009) or regional white matter density (Hulshoff Pol, et al., 2006) in twins.

New imaging techniques like DTI are showing evidence that white matter integrity (axonal membrane integrity, myelin etc.) changes according to environmental experience (Fields, 2008). It is important to see how this information can fit with the twin imaging studies. If we regard myelination as a developmental process, it could be expected that environment plays a big role in the variability of the white matter integrity as measured by DTI, knowing that myelin, although not necessary, is a modulator of fractional anisotropy (FA). However, results regarding the heritability of white matter integrity as measured by FA are inconsistent. Some studies showed that FA is under strong genetic control (explaining almost 80% of the variance) (Chiang, et al., 2009; Kochunov, et al., 2010) while others reported significant genetic effects on radial and longitudinal diffusivities only (Brouwer, et al., 2010). Some also noted that heritability of FA is greater in adolescence versus adulthood (Chiang, et al., 2010). Though, what is consistent is that diffusion studies showed that regardless of the diffusion measure analysed, the highest heritability for white matter integrity is found in bilateral frontal and parietal brain regions and corpus callosum.

When investigating variations in local white matter volumes, studies reported high control of common environmental factors (Brun, et al., 2009), with high genetic influence being limited to a few brain areas only. Nevertheless, two regions are consistently reported to be under high genetic control both in terms of white matter integrity and volume, and these are the superior fronto-occipital fasciculus (Brouwer, et al., 2010; Hulshoff Pol, et al., 2006; Peper, et al., 2009) and the corpus callosum (Brouwer, et al., 2010; Brun, et al., 2009; Hulshoff Pol, et al., 2006; Peper, et al., 2009; Pfefferbaum, et al., 2000; Scamvougeras, et al., 2003) in both paediatric and adult twin samples. Furthermore, heritability of corpus callosum macrostructure and microstructure demonstrated significant (and differential) genetic regulation even in old age - with anterior interhemispheric connecting pathways more susceptible to environmental influences (Pfefferbaum, et al., 2001). This is consistent with Brun et al. (2009) who noted that frontal lobe white matter is more environmentally driven, compared to posterior brain regions. On the other hand, environmental factors were found to be dominant for white matter density in the orbitofrontal cortex, anterior cingulate, and (parts of) the cingulum (Peper, et al., 2009).

Brain morphometric measures

As noted above, up to now, many studies have reported strong genetic effects on brain size, but factors affecting brain shape are still poorly understood. In general, it seems that brain morphometry is heritable, but to a lesser extent than brain volume, with gyral and sulcal patterns appearing more similar in MZ compared to DZ twins (Schmitt, et al., 2007). Deep sulci appear to be more genetically determined than superficial sulci (Biondi, et al., 1998; Lohmann, et al., 1999). But overall, cortical gyral and sulcal patterns, though significantly affected by genes, seem to be determined primarily by environmental factors (Wright, et al., 2002).

Functional imaging measures

Functional MRI experiments on twins are extraordinarily rare with only nine reports published to date. Four studies have examined the heritability of brain activation during working memory tasks and found a strong genetic influence accounting for up to 80% of the total variance in BOLD response during working memory tasks (Blokland, et al., 2008; 2011; Karlsgodt, et al., 2010; Koten, et al., 2009). Similarly, high heritabilities were observed for the measures of default-mode activity (Castellanos, et al., 2010; Glahn, et al., 2010) and functional network cost-efficiency (Fornito, et al., 2011). The only study that found no genetic effects measured by fMRI was reported by Côté, et al. (2007) who examined the neural substrates of sadness in 8-year-olds. No genetic influences on the relationships between sadness and brain activation were found in two areas of the brain (medial prefrontal cortex and ventrolateral prefrontal cortex) previously correlated with the subjective experience of sadness. These relationships were dominated by unique environmental effects. Taken together, fMRI research on twins suggests that brain activation can be both strongly genetically or environmentally influenced. However, it should be emphasised that most functional MRI studies on twins suffer from very small sample sizes by the standards of quantitative genetic research (Fornito, et al., 2011; Karlsgodt, et al., 2007; Spaniel, et al., 2006) which can bias the results.

Cerebral asymmetry

There are reports of cerebral asymmetry with regard to heritability estimates of brain structure. However these reports are inconsistent and appear to be sensitive to the choice of volumetric measure used. Overall, there are findings of no asymmetry (Wright, et al., 2002) or the left hemisphere being under greater genetic (Joshi, et al., 2011; Lohmann, et al., 1999; Pell, et al., 2009; Tramo, et al., 1995; Thompson, et al., 2001; Yoon, et al., 2010) or environmental control (Geschwind, et al., 2002; Carmelli, et al., 2002).

There are scarce data on the genetic control of known cerebral structural asymmetries. Only one diffusion study so far has examined the genetics of the brain fibre asymmetries (Jahanshad, et al., 2010). They showed that genetic factors accounted for approximately one third (20-37%) of the variance in asymmetry of white matter pathways such as inferior fronto-occipital fasciculus, the anterior thalamic radiation, forceps major and uncinate fasciculus in 374 adult twins. Shared environmental factors accounted for between 10-15% of the variance in asymmetry for the corticospical tract and the forceps minor. The rest of the variance was attributed to unique environmental effects, which seemingly played the biggest role in defining the fibre asymmetries. It should be noted however, that studies in fibre-level asymmetries may be confounded by known asymmetries in shape, such as the natural petalias that make the right frontal lobe protrude beyond the left (Jahanshad, et al., 2010).

4.1.1.2 Final remarks

On the basis of the twin and family MRI studies it can be inferred that genes play a highly significant role in the generation of the variability in global brain volumes, particularly for larger structures (Baaré et al., 2001; Pennington et al., 2000; Pfefferbaum et al., 2000; Schmitt, et al., 2007). Notwithstanding some differences in findings, it seems that individual variation in brain areas involved in attention, language, visual processing as well as sensory motor processing are strongly genetically influenced (Peper, et al., 2007). The next section will introduce language-related research and discuss how we came to understand the heritability of language processing in the human brain.

4.1.2. Heritability of speech and language disorders

Systematic analyses of language and speech disorders have shown that these are heritable and importantly that most patients with these disorders exhibit abnormalities in brain structure. We cannot deduce from this that language-related structures are under substantial genetic control, but it is nonetheless worthwhile to discuss the research on language impairments further. However, as with other genetic analysis of developmental traits, considerable challenges are present since speech, language, reading and other language-related abilities change significantly with advancing age.

Disorders of communication, consisting of speech and language disorders, can either occur alone, in otherwise normal developmental trajectory (primary disorders), or can be part of the global developmental deficits, such as in learning disability, autism spectrum disorders (see Chapter 6.1.2), hearing impairments etc. Primary speech and language disorders are classified into five distinct categories (DSM-IV) (Newbury and Monaco, 2010):

- expressive language disorder (LD);
- mixed receptive-expressive LD;
- phonological disorder (disrupted production and proper use of speech sounds);
- stuttering (disrupted fluency), and
- communication disorder not-otherwise-specified.

Newbury and Monaco (2010) describe disorders of speech as impairments in the production of intelligible speech that include stuttering, phonological disorder, and developmental verbal dyspraxia (impairment in the coordination and motor control of the speech organs). On the other hand, disorders of language are more subtle and include problems with the correct formation of words (morphology) or sentences (syntax), the derivation of meaning (semantics), the use of linguistic context (pragmatics) which may affect expressive and/or receptive language as well as non-verbal language (e.g. reading and writing - developmental dyslexia) (Newbury and Monaco, 2010).

In order to explore whether there are substantial genetic influences on communication disorders we can investigate whether disorders run in families. Recent studies show that speech and language deficits show strong familial aggregation (Barry, 2007; Lewis, 2007) mostly due to genetic factors (Bishop, 2002; Felsenfield, 2000; Spinath, et al., 2004). However, that is not the complete story. Research reveals that shared environmental contributions are also substantial (Beijsterveldt, et al., 2010; Spinath, et al., 2004). It is now considered that genetic mechanisms underlying susceptibility to speech and language disorders are multifactorial in nature, involving complex interactions between genes and environmental factors (Newbury and Monaco, 2010). I will discuss in more detail specific language disorders.

Specific Language Impairment

Specific language impairment (SLI) is used as an umbrella term for expressive LD, mixed receptive-expressive LD and sometimes phonological disorder, and presents an unexpected failure to develop age appropriate oral language. There is sufficient evidence that SLI is highly heritable: relatives of language-impaired individuals are at increased risk of developing SLI, and family members frequently report literacy difficulties (Stromswold, 1998, 2001). Furthermore, twin studies consistently point to a strong genetic role in susceptibility to SLI, repeatedly showing that monozygotic twin pairs are linguistically more similar to each other than dizygotic twins (Bishop, 1995; Hayiou-Thomas, 2008; Lewis and Thompson, 1992; Newbury, et al., 2005; Tomblin and Buckwalter, 1998; Viding, et al., 2004). Nevertheless, the inheritance pattern is hardly unambiguous. One exception is the case of the KE family, where a single autosomal mutation is associated with a distinctive pattern of this speech-language disorder (Lai, et al., 2001). The rest of the studies on SLI show inconsistent heritability patterns. There are two main reasons why this might be so.

First, SLI is clinically heterogeneous, hence, different heritability estimates can be the consequence of different diagnostic criteria and psychometric tests used. Some studies indicate that strong genetic influence, on both structural and pragmatic language impairments in children, can be detected equally well with psychometric tests as well as simple checklist for communication skills completed by parents or teachers (Bishop, et al., 2006). However, although the majority of studies reported heritability of 0.5 or more, a recent report from Twins Early Development Study (Hayiou-Thomas, et al., 2005) found only a minor genetic influence in 4-year-olds. When differences in studies were analysed, it was noted that substantially higher heritability was observed if SLI was defined in terms of referral to speech and language pathology services rather than language test scores (Bishop and Hayiou-Thomas, 2008). The fact that heritability estimates of specific language impairments seem to depend on diagnostic criteria used should therefore be taken into account when summarizing results of different studies.

Second, because of clinical heterogeneity, different deficits within SLI can have different aetiological origin, hence heritability estimates might vary for different aspects of SLI. For example, it has been suggested that low-level auditory deficits cause phonological problems in SLI. However, a twin study showed that these deficits have quite different origins, with environmental factors more important for auditory deficit, and genes more important for deficient phonological short-term memory (Bishop, et al., 1999). Likewise

morphosyntactic deficits in SLI are also highly heritable, but have different genetic origins from impairments of phonological short-term memory (Bishop, 2006). A genetic perspective shows that SLI is a complex and heterogeneous disorder, likely to be guided by multiple genes that interact, both with each other and with the environment to produce an overall SLI susceptibility and phenotype. Some studies point to the involvement of chromosomes 16q and 19q in the condition (SLI Consortium, 2002, 2004). However, due to the lack of clear genotype–phenotype relationships molecular genetic studies have been hindered, and a clear genetic basis of the condition is still not known.

Importantly, brain abnormalities were found in patients with SLI, especially in the language-related areas of the left hemisphere. Gauger, et al. (1997) reported a volume decrease in the left pars triangularis as part of the Broca's area in 9-year-olds, while Jernigan, et al. (1991) found a volume decrease in the left posterior temporal region. Furthermore, children with SLI seem to have a loss of left structural asymmetry for frontal and temporal language-related regions (Gauger, et al., 1997; Plante, et al., 1991).

Dyslexia and reading difficulties

Traditionally, SLI and developmental dyslexia (or reading disability) have been regarded as separate disorders. However, there is a growing recognition that problems with oral and written language frequently co-occur, and genetic factors that influence variation in SLI were found to account for much of the relationship between early speech and later reading (Hayiou-Thomas, 2008). Today many experts regard SLI and dyslexia as different manifestations of the same underlying disorder (Newbury, et al., 2005). It is estimated that for both disorders the prevalence in first-degree relatives of affected individuals is 30–50%, compared to the 5–10% prevalence in the general population (Barry, et al., 2007; Fisher and DeFries 2002). Research showed that reading difficulties are both heritable and stable (Astrom, et al., 2011, 2007). Genetic control varied from moderate, for general reading backwardness or specific reading retardation (Stevenson, 1987), to strong, for general reading disability (Hensler, et al., 2010; Kirkpatrick, et al., 2011). Importantly, individuals with developmental dyslexia or dysphasia often present with structural brain abnormalities, such as polymicrogyric cortex in language-related areas, around the left perisylvian fissure (Spalice, et al., 2009).

Nonspecific Language Impairment

Nonspecific language impairments (NLI), compared to SLI, do not occur in isolation, and children exhibit both verbal and nonverbal deficits. It is acknowledged that there is a distinction between specific (SLI) and nonspecific (NLI) language impairment at an etiological level (Hayiou-Thomas, et al., 2005). For children with NLI genetic control on language impairment was moderate while shared environmental influence was substantial. A similar pattern was found for SLI, although there was a trend for the genetic effects to be smaller for SLI than for NLI.

Stuttering

Behavioural genetic studies of speech fluency have focused on participants who present with clinical stuttering. Research showed moderate and almost equal involvement of genetic and shared environmental factors. In a study done by Beijsterveldt and colleagues (2010) genetic analyses revealed heritability estimates of 0.42 for stuttering and 0.45 (out of 1.00) for high non-fluency. Shared environmental factors were also significant, explaining 0.44 of the individual differences in probable stuttering and 0.32 in non-fluency.

4.1.2.1 Identification of the candidate genes: molecular genetics approach

Twin and family studies have showed that genetic influences should not be underrated in the aetiology of language and speech disorders, however progress in identifying genes has been slow. Most of the candidate genes for dyslexia and SLI have been discovered in family-based samples through genetic association studies which targeted chromosomal regions previously mapped by linkage studies (Parrachini, 2011). Identified candidate genes for specific language impairment (SLI) and dyslexia include FOXP2, FOXP1, CNTNAP2, DYX1C1, SRPX2, and others. These will be discussed in more detail bellow, focusing on associations with brain abnormalities.

FOXP2 and FOXP1

The link between transcription factor forkhead box protein P2 (FOXP2) and language was first recognised in the large three-generation KE family, whose members are disproportionately affected by language impairments (Lai, et al., 2001). Structural and functional brain imaging of individuals with FOXP2 mutations shows volume and activation differences during language tasks, particularly in cortico-cerebellar and cortico-striatal circuits (Fee and Scharff, 2010). Today it is evident that this gene is important for development of brain regions responsible for fine motor control (motor cortex, striatum, and cerebellum) and that its disruption has severe consequences on development of speech (Newbury and Monaco, 2010). Moreover, research in songbirds and juvenile zebra finches noted that this gene is implicated in auditory-guided vocal motor learning (Fee and Scharff, 2010). Hence, it is possible that FOXP2 is not only important for early brain development, but it might also play a role in post-developmental formation of language-related circuits. Similarly to FOXP2, forkhead box protein P1 (FOXP1) seems to have overlapping functions during brain development. FOXP2 and FOXP1 work together throughout tissue development, thus it is likely that they are both involved in biological processes important for development of speech and language (Newbury and Monaco, 2010).

CNTNAP2

Common variants in contactin-associated protein-like 2 (CNTNAP2), a neurexin superfamily member, may play a role in susceptibility to language impairments and autism. Research gave evidence of CNTNAP2 effects on developing language areas of the frontal and temporal lobes.

CNTNAP2 shows asymmetric patterning in the brain, with anterior-enriched cortical expression. Its mRNA is significantly elevated in the developing human brain in the frontal and temporal lobes (Abrahams, et al., 2007), regions responsible for supporting speech and language learning and processing. In addition, CNTNAP2 is believed to be important for the construction of neural circuits, since Caspr2, the protein encoded by CNTNAP2, is thought to assist in interactions important for cellular migration and laminar organisation (Scott-Van Zeeland, et al., 2010*).* Structural MRI showed that individuals who carry two copies of the genetic "risk" variants have significantly reduced volumes of gray and white matter across several brain regions including prefrontal cortex, fusiform gyri, occipital cortices, and cerebellum (Tan, 2010). Furthermore, functional neuroimaging studies noticed a relationship between frontal lobar connectivity and common genetic variants in CNTNAP2, implicating dysfunction of long-range connections within the frontal lobe in autism (Scott-Van Zeeland, et al., 2010)

DYX1C1 and SRPX2

The status of dyslexia susceptibility 1 candidate gene 1 protein (DYX1C1) as a susceptibility gene for developmental dyslexia is unclear. It was found that missense mutation rs17819126 of DYX1C1 gene influences reading and spelling ability with additional effects on short-term information storage or rehearsal (Bates, et al., 2010). Another gene implicated in developmental dyslexia is sushi repeat-containing protein (SRPX2) gene. Mutations of the SRPX2 have been associated with brain structural abnormalities, such as bilateral perisylvian polymicrogyria that often accompanies developmental dyslexia (Spalice, et al., 2009). These observations suggest a role for DYX1C1 and SRPX2 in the development and functioning of language-related areas in humans.

4.1.2.2 Final remarks

The last decade has seen an eruption of research aimed at deciphering the genetic basis of speech and language disorders. The identification of FOXP2 triggered novel investigations on heritability of language and speech foundations, but in comparison to other developmental disorders with a genetic contribution (e.g. autism), speech and language disorders are still somewhat understudied. Many of the studies described above need to be replicated in independent cohorts, since they involve rather small samples. Furthermore, correlating genetic variation with specific phenotypic features is extremely difficult, since associations alone can never prove causality. As our understanding of phenotypes becomes more refined, we may be able to identify different subtypes with distinct aetiologies, which might aid our research into the genetic basis of communication disorders. Up to now, research suggests that language impairments are heritable. Having said that, the next step is to investigate the implications of these findings on the genetic basis of normal variations in language skills. The next section will discuss the heritability of language-related skills in a healthy population.

4.1.3. Heritability of normal variations in language skills - behavioural genetic approach

Unconsciously, we tend to perceive language as a whole, although it is essentially a manifestation of many different modalities and components. An apparently simple task of producing a sentence demands many distinct and specialized anatomical mechanisms and sub-mechanisms, some essential and others with a smaller, supportive, role. Linking different language modalities to various brain regions has been an ongoing venture for almost two centuries. Although our study is concerned with the heritability of 'language anatomy', and not of functional properties expressed by different language modalities, I will briefly discuss the behavioural findings of normal variation in language skills.

Psychology, sociology and linguistics, when gathered together under the umbrella term of the behavioural sciences, provide useful cues to the heritability of different aspects of language. The main branches of behavioural genetic analyses focus, for example, on reading and writing skills, phonology, grammar (morphology, syntax), lexical abilities (vocabulary) and so on. At the present time there is no one-to-one correlation between neuroimaging and behavioural genetic data, because there are no studies that have matched both methods. Analyses of subjects in neuroimaging have, thus far, proceeded along very separate lines from the analyses in behavioural genetic studies.

This section will try to answer, by studying the results of behavioural genetic studies, whether heritable factors are responsible for the variation in linguistic abilities observed among healthy people, or do heritable factors only account for the variance observed for people diagnosed with language disorders mentioned in the previous section. I will focus on observing how normal variations in language skills are driven by innate genetic mechanisms matched with the experience of specific and shared linguistic environments.

Evidence of the importance of genetic factors in normal variation of language skills comes from Stromswold's (2001) meta-analysis of almost 100 twin studies. Her results indicate that genetic factors play an important role in the variation of the rate of language acquisition and linguistic proficiency attained by both children and adults. Depending on what aspect of language is assessed, heritable factors accounted for 1/4 to 1/2 of the variance in normal twins' linguistic abilities. However, this was a much lower figure compared to language-impaired twins where heritable factors accounted between 1/2 to 2/3's of the variance. Based on this meta-analysis, it seems that genetic factors account for much of the variance in linguistic abilities among people with written or spoken language disorders but much less of the variance in linguistic abilities among the healthy population.

Studies suggest that different genetic factors are involved in different aspects of language (e.g. written language vs. spoken language; lexical vs. syntactic abilities). This is further complicated by the fact that one aspect of language can be analysed in different ways. For example, analyses of reading related skills could include phoneme awareness, word recognition, phonological decoding, and/or orthographic coding tests. Strong genetic and negligible-to-small environmental influences were found to affect normal variation in

reading-related skills, such as reading comprehension (Olson, 2011), reading span (Kremen, et al., 2007) word recognition (Kremen, et al., 2007; Olson, 2011), phonology (Hohnen and Stevenson, 1999), phonological awareness (Olson, 2011), literacy (Hohnen and Stevenson,1999) and reading ability (Hensler, et al., 2010; Kirkpatrick, et al., 2011), but also speech-related skills (Hayiou-Thomas, 2008; DeThorne, et al., 2008), writing and spelling (Olson, 2011). Moderate genetic effects were observed for phonology (Kovas, et al., 2005), articulation (Kovas, et al., 2005), grammar (Dale, et al., 2010; Hayiou-Thomas, 2008; Kovas, et al., 2005), vocabulary (Dale, et al., 2010; Hart, et al., 2010; Hayiou-Thomas, 2008; Kovas, et al., 2005), verbal memory (Kremen, et al., 2007; Kovas, et al., 2005) and reading comprehension (Hart, et al., 2010). Hence, heritability estimates of language skills depend on the specific language measure used. Overall, research results indicate that for both language-impaired and normal twins, genetic factors play a greater role for phonological and syntactic abilities than for lexical abilities (Stromswold, 2001). Heritable factors account for about a third of the variance in normal twins' lexical abilities and about a half of the variance in their phonological and syntactic skills. Consistent with Stromswold's (2001) meta-analytic results, some years later Stromswold, et al (2005) published a study on language-impaired and normal twins, showing that genetic factors account for more of the variance for phonology (70% for language-impaired and 31% for normal twins) and syntax (100% for language-impaired and 26% for normal twins) than for vocabulary (69% for language-impaired and 5% for normal twins).

Nevertheless, two measures were consistently found to be under a greater shared environmental effects: vocabulary knowledge (Hart, et al., 2009; Hayiou-Thomas, 2008; Olson, 2011) and grammar skills (Hayiou-Thomas, 2008). Thus, there seems to be a stable relationship between home literacy environment and vocabulary and grammar. If we monitor a child's development, we know that vocabulary growth starts slowly between 12 and 18 months of age (Karmiloff-Smith, 2004). However, at around 21 months of age many infants show a 'vocabulary spurt' where the rate of vocabulary growth increases dramatically. From then on, the transition from early child-like word combinations to full blown grammar is rapid. By the time children reach their fourth birthday, they have triumphed over an impressive range of grammatical tools, they can make statements, ask questions and issue commands (Karmiloff-Smith, 2004). Likewise, longitudinal investigations have indicated that a richer early home literacy environment is associated with enhanced vocabulary ability in early and middle childhood (van Steensel, 2006) and adolescence (Olson, et al., 2011). These studies also pointed to the importance of developmental stage when studying heritability. Thus, the next section will briefly discuss the relationship between age and heritability of language.

Heritability of language skills and age

We have seen that heritability estimates of variation in language skills are often different for children compared to adults. This is not surprising when we know that the influences of genes and environment change over lifetime, and that language skills expand in both quantitative and qualitative ways during development. Hence, it is important to understand the ways in which age can influence language heritability estimates. We know that some traits exhibit a linear age-related increase in heritability, such as general cognitive ability *g* (Haworth, et al., 2010) and IQ (Bouchard, 1998). When observing heritability of different

aspects of language, the picture is somewhat different due to immense complexity and variety of language skills. Previous behavioural genetic analyses of twin data have suggested a possible developmental dissociation between genetic influences on word reading and spelling deficits as a function of age (overcoming reading but not spelling problems over time) (Friend, 2007). Research shows that genetic influence declines across age for word recognition, and increases for spelling recognition (DeFries, et al., 1997; Wadsworth, et al., 1989). This pattern of decline in heritability across age for reading and increase for spelling conformed to that predicted by the developmental dissociation hypothesis. In conclusion, research shows that age is an important factor that should not be ignored in behavioural genetics, since the effects of age can alter significantly heritability estimates. However, there are also other factors that can affects the heritability estimates of language skills, and these will be briefly discussed in the following section.

Why are identical twins linguistically different?

Although the heritability estimates obtained in twin studies indicate that MZ co-twins are more linguistically similar than DZ co-twins, heritability estimates for language in healthy twins rarely exceed 60% and MZ twins (who are usually assumed to have identical genetic and shared environmental contributions) sometimes have very different linguistic profiles (Stromswold, 2006). Some argue that this is because twins are more likely to suffer linguistic delays and impairments than singletons - which makes MZ twins linguistically different and therefore lowers the heritability estimate. Postnatal factors, such as differences in linguistic input which twins receive, are usually assumed to be the major reason for these findings. However, Stromswold (2006) argues that perinatal environmental factors (premature birth, low birth rate, placental and amniotic complication, intrauterine infection) affect linguistic development more than postnatal factors, while postnatal factors affect cognitive development more than perinatal factors. This, in Stromswold's view, is because perinatal factors are principally biological, whereas postnatal factors tend to be psychosocial (e.g., how and how much parents speak to their children). Overall, the contributions of both perinatal and postnatal factors are significant in making identical twins linguistically different, nonetheless the classical twin study design does not have the power to discern between the two. Hence, other methods are needed to shed the light on specificities of what makes identical twins linguistically different.

Conclusions

So far, research has given us reasons to believe that most of the language skills are under high genetic control. What does this mean for brain correlates of language function? Posthuma, et al. (2003) have compared the similarity of twins' test performance and twins' brain structures. The verbal comprehension subcomponent of full scale IQ, which was highly heritable, was not related to grey matter, white matter, or cerebellar volume either genetically or environmentally. This result is surprising, but as they suggested, it most likely reflected the fact they used overall brain volumes rather than regional brain volumes. Due to the lack of other, more regionally specific studies, we cannot infer about one-to-one correlation, though behavioural genetic approach would be a very useful companion to neuroimaging studies. The next section will focus on brain imaging studies, in order to understand the heritability of language brain regions.

4.1.4. Heritability of variation in language areas and language lateralisation

Numerous studies have embarked on investigating the inheritance of grey and white matter brain regions that subserve language processing. Research has examined both regional language-related brain areas, and the functional and anatomical lateralisation pattern specific to language function. This section will try to give a summary of recent key findings in the field of language-in-the-brain heritability, in order to provide a context for understanding the importance of mapping the heritability of specific perisylvian language pathways (which is the aim of my study).

4.1.4.1 Heritability of variation in perisylvian language areas

Structural MRI findings

Structural volumetric and morphometric imaging studies, along with diffusion tensor imaging studies, have examined the effects that the interplay between genes and environment have on language-related brain regions at different points in human life. In general, findings report significant heritability of frontal, temporal and parietal brain regions in both paediatric (Lenroot, et al., 2009) and adult twin data (Joshi, et al., 2011; Hulshoff Pol, et al., 2006; Thompson, et al., 2001).

Genetic brain-mapping techniques applying structural MRI (sMRI) data from twins, indicate that grey matter volumes in perisylvian areas are under tight genetic control and are highly heritable (Toga and Thompson, 2003). A review of 17 sMRI and CT brain imaging twin studies highlights moderate to high heritability for grey matter densities of Broca's area (Peper, et al., 2007). This is in line with a study by Thompson, et al. (2001) that found significant genetic effects on cortical structures in both Broca's and Wernicke's language areas. Near-identity was found in MZ twins, and 90-100% correlation in DZ twins in perisylvian language cortices including supramarginal, angular territories, and Wernicke's area. Furthermore, language asymmetry reflected heritability asymmetry, with highly significant heritability on the left (but not right) of Broca's and Wernicke's area. Similarly, cortical thickness in Wernicke's area showed a significant genetic effect in a later study by Joshi, et al. (2011) and was, in addition, correlated with full-scale IQ. Contrary to Thompson, et al. (2001) findings, Hulshoff Pol and colleagues (2006) did not observe significant heritability for Broca's language area. The authors suggested that the differences in their findings might be due to different transformation and structural equation modelling procedures. Thompson, et al. (2001) applied twice the difference between MZ and DZ intraclass correlation coefficients and a permutation analysis to correct for multiple comparisons while Hulshoff Pol, et al. (2006) used random field theory for multiple comparisons correction. Overall though, there is substantial evidence that grey matter cortices underlying language processing are under tight genetic control.

Brain morphometry also appears to be significantly heritable, though to a lesser extent than volume (Schmitt, et al., 2007). Some familial influences, but also a role of the unique environmental effects, were observed in the development of gyral (Biondi, et al., 1998) and sulcal patterns (Lohmann, et al., 1999). Although gyral–sulcal forms appear to be much less heritable, it seems that individual variation in morphology of language processing areas is strongly genetically influenced. The gyri with the highest heritability estimates are the ones underlying language, speech and social cognition; functions thought to have developed relatively recently in evolutionary time (Lenroot, et al., 2009). Schmitt, et al. (2008) observed that during childhood the regions with the highest heritability included language-related gyri such as inferior frontal gyri, left medial frontal gyrus, the pre- and postcentral gyri, left supramarginal gyrus, and the left inferior temporal gyrus. Likewise, Yoon et al. (2010) found significant genetic effects for the left middle and inferior frontal gyri and precentral gyri in a paediatric twin sample. However, the genetic effects seem significant even in adulthood. Lenroot, et al. (2009) found significant heritability in the postcentral and supramarginal gyri in both childhood and adolescent twin data. Similarly, Hulshoff Pol, et al. (2006) found high heritability of left postcentral gyrus and Heschl's gyrus bilaterally, in an adult twin sample. However, these positive findings were not replicated in a study of young adult twins by Joshi, et al. (2011), where only a trend towards significant genetic effects was observed in the mentioned gyri. Although there are some inconsistent results within studies, a recent review of brain imaging studies in twins points to high heritability estimates for both Heschl's gyrus and postcentral gyrus (Peper, et al., 2007) further highlighting the relevance of these brain areas when searching for genes influencing language-related brain structure and language function.

The only study thus far that has come close to examining the heritability of the arcuate fasciculus/superior longitudinal fasciculus (SLF) applied volumetric MRI and voxel-based morphometry on a sample of 45 MZ and 62 DZ paediatric twins (Peper, et al., 2009). High heritability was observed for white matter density in SLF and gray matter density of frontal and temporal brain areas. Genetic effects were significant in the SLF bilaterally, with heritability estimates ranging from 76 to 91%. Reported heritable white matter voxels within the SLF largely overlapped with the post-mortem maps of fibre bundles supporting the notion that this fibre tract is involved. However, since no actual fibre-bundles could be traced on the T1-weighted brain images, we have to interpret SLF results with some caution since location of SLF cannot be categorically stated. Diffusion imaging findings can thus provide more insights into the heritability of specific white matter tracts, such as the arcuate fasciculus.

Diffusion MRI findings

Knowing that experience changes white matter and diffusion characteristics (Bengtsson, et al., 2005; Fields, 2008) and that myelination is a developmental process that continues into the third decade of life (Yakovlev and Lecours,1967) it might be expected that the effects of environment will play a bigger role in shaping perisylvian white matter pathways as compared to genes. Diffusion tensor imaging (DTI) findings show that the arcuate fasciculus in not fully mature in early human ontogeny, at least not in 7-year-old children compared with adults as measured by fractional anisotropy (Brauer, et al., 2011). Is it plausible that

the environment is driving this continuing maturation of perisylvian pathways? Although the numbers of DTI studies are modest, their results suggest otherwise. Fibre architecture in most major white matter structures is found to be highly heritable, consistent with prior reports that brain morphometry is also highly heritable (Toga and Thompon, 2003). Advances in quantitative genetics has led to three DTI studies focused on elucidating the amount of genetic influence on the variability of the arcuate fascicululs (referred to as SLF in the mentioned studies) through analysis of fractional anisotropy, axial and radial diffusivities in healthy twins (Brouwer, et al., 2010; Chiang, et al., 2009; Kochunov, et al., 2010).

Chiang, et al. (2009) explored white matter integrity, quantified using fractional anisotropy (FA) in 92 identical and fraternal adult twins. Genetic factors explained almost 80% of the variance in global white matter integrity. Shared environmental effects were detected but were not significant in the left temporal lobe, and the effects of unique environment were relatively small. Importantly, significant contributions of genetic factors were found in the SLF bilaterally. That the heritability of FA measured in SLF is highly heritable was confirmed in a later study by Kochunov, et al. (2010). They used a larger sample of 467 adult subjects and applied the TBSS method (Smith, et al., 2006) to perform whole brain heritability analyses together with the analysis of ten major white matter tracts. Kochunov, et al. (2010) concluded that the microstructure of cerebral white matter is under a strong genetic control, with SLF showing significant heritability for FA measures ($h^2 = 0.58$), while radial and axial diffusivity measurements in SLF were not significant. However, TBSS technique does not allow us to isolate and identify, categorically, the SLF, so these results should be taked with some caution. Contrary to Kochunov, et al. (2010), Brouwer, et al. (2010) performed fibre tractography and observed that FA measured in SLF was not significantly influenced by genetic factors in 185 paediatric subjects. In contrast, when studying axial and radial diffusivity separately, significant genetic effects were observed for both in the right SLF. Presence of genetic influence was most widespread for radial diffusivity, for which significant influences of genetic factors were found in the SLF bilaterally (left SLF $h^2 = 0.64$). Significant influence of genetic factors on variation in axial diffusivity was found in the right SLF ($h^2 = 0.35$). Genetic factors influencing magnetization transfer ratio (MTR), and thus possibly myelination, were also pronounced in the SLF bilaterally. These results are supported by a voxel-based morphometry (VBM) study in this sample (Peper, et al., 2009), which showed moderate to high heritability estimates of white matter density in areas covering the SLF.

In summary, at the time of writing this dissertation, DTI studies have just started applying quantitative genetics to study the inheritance of white matter pathways. Although this area is in its infancy, the results reached so far show that genetic effects play an important role on diffusion measures of the perisylvian language pathways. However the magnitude of these effects varies with age, so that the heritability of FA, a measure of microstructural directionality, in SLF is higher in adulthood compared to childhood while the opposite is true for the measure of axial diffusivity. Nevertheless, no study to date has examined the heritability of the three segments of the arcuate faciculus, and/or compared the heritability estimates of diffusion measures versus volumetric measures in these pathways. This is of importance because different segments of the arcuate fasciculus might have different vulnerability to the environmental stressors, which would have clinical implications, and would furthermore imply that different genetic mechanisms are driving

their development. Besides investigating heritability of specific segments of the arcuate fasciculus, this PhD study also examined the heritability of the language lateralisation patterns. Hence, the next section is dedicated to introducing the genetic basis of language lateralisation.

4.1.4.2 The genetic basis of language lateralisation

It is well-known that left and right cerebral hemispheres differ both functionally and anatomically in respect to language processing (see Chapter 1). The frequency distribution of language lateralisation reveals that approximately 90% of humans are left-hemisphere dominant (Knecht, et al., 2000). However, little is known about the environmental or genetic factors that govern this asymmetry bias for processing language. The inheritance of brain asymmetries is difficult to research and findings tend to be inconsistent. This section will try to elucidate the hereditary mechanisms that drive functional and anatomical lateralisation of language, deriving explanations from molecular genetic, twin and family studies.

One vital question concerning the asymmetry of the perisylvian language network is at what stage of life can this left-hemispheric asymmetry be observed? Several findings note that the arcuate fasciculus shows an early asymmetry towards the language-dominant hemisphere already in infants (Dubois, et al., 2009) but these white matter connections are not fully mature until very late in human life (Giorgio, et al., 2008). This developmental pattern might suggest that both genes and environment are important for the arcuate fasciculus, although at different points in time. Strong genetic effects are more likely present during early years, in order to precipitate the language asymmetry, but significant environmental influences act at a later stage and mould the final outcome of maturation process.

Molecular genetic studies

Recent advances in molecular genetic research point to large differences in genetic make-up between the left and right hemispheres in humans by investigating specific gene effects on developing language areas of the frontal and temporal lobes. Although studies are scarce, an understanding of the developmental pattern of the perisylvian language regions in the human brain is starting to emerge. It has previously been noted that cortical asymmetry of these regions has a strong molecular basis demonstrated through asymmetrical gene expression in perisylvian cortex at early human embryonic stages (Abrahams, et al., 2007; Geschwind, et al., 2001; Sun, et al., 2005).

Abrahams, et al. (2007) performed a genome-wide analysis of human perisylvian cerebral patterning during mid-gestation, a critical epoch in cortical regionalization, and found 345 genes asymmetrically expressed between superior temporal gyrus (STG) and the remaining cerebral cortex. They found LIM domain-binding 1 (LDB1) gene - a regulator of LMO4 gene, to be enriched in STG. LMO4 gene is involved in asymmetrical patterning and is consistently more highly expressed in the right perisylvian human cerebral cortex than in the left (Sun, et al., 2005). In addition, contactin associated protein-like 2 (CNTNAP2), of which mutations are known to be associated with language delay and autism, shows a remarkable pattern of

anterior-enriched cortical expression. CNTNAP2 expression is high in anterior temporal and prefrontal regions in humans, but low or absent in rodents (Sun, et al., 2005).

Molecular genetic studies indicate that a strong genetic control is driving early language lateralisation. It seems that gene expression asymmetries mirror the asymmetries of language functional and anatomical organisation. However, these studies cannot answer the question of whether this genetic control persists in later years, when language dominance is already formed and language skills have developed. To answer this question, we need to turn to twin and family studies of both paediatric and adult data.

Twin and family studies

There must be many ways in which genetic and non-genetic influences combine to determine functional and anatomical language asymmetries. The task of this subsection is to try to elucidate the formula by investigating twin and family studies (for more details on the twin and family research methods see the following section 3.1.5). These studies, of strength of functional and anatomical lateralisation in twins and families, suggest only minor genetic effects in both paediatric and adult data.

Heritability of variation in functional language lateralisation

The first twin and family studies related to the genetics of language lateralisation examined the functional asymmetry in the human brain. The reason behind this is that functional language organisation could be easily explored using behavioural tests such as dichotic listening, long before the advent of non-invasive imaging methods. A standard dichotic listening test involves simultaneously presenting two different auditory stimuli to both ears, and then asking the participants to identify one or both. This test elicits a right ear advantage in most right-handed subjects, reflecting left-cerebral dominance for language processing (Kimura, 1967). With the advent of non-invasive imaging methods new findings emerged unmasking the underlying neurobiology with the functional imaging method. However, there is still insufficient information on language lateralisation in twins and their relatives. Studies assessing language lateralisation in twin pairs used small samples and it is therefore difficult to generalise their results to the rest of the population (Anneken, et al., 2004; Springer and Searleman, 1978; Jäncke and Steinmetz, 1994; Steinmetz, et al., 1995; Sommer, et al., 2004).

It is important to mention that recent brain imaging studies have demonstrated that an early functional asymmetry is already present in the first months of life (Dehaene-Lambertz, 2000; Dehaene-Lambertz, et al., 2002). Hence, it is highly possible that strong familial (genetic and shared environmental) effects are driving this asymmetry. Dehaene-Lambertz, et al. (2010) noted that the mother's voice plays a special role in the early shaping of the posterior language areas at the level of the planum temporale. From this we can infer that shared environment plays an important role in functional language maturation.

The picture is less clear when exploring genetic effects on asymmetry. An early study by Bryden (1975) used a dichotic listening test in 49 families to study the inheritance of brain asymmetries responsible for language processing. Speech lateralisation showed small correlations between parents and children, and no correlation at all between siblings - suggesting only negligible genetic effects. Conversely, a later study by Anneken, et al. (2004) found a strong association for strength of language lateralisation between relatives using functional transcranial Doppler ultrasonography (fTCD) in ten families. This technique measures a localised cerebral blood flow velocity change due to neural activation during language tasks. The results showed that in families were both parents were strongly lateralised children tended to be strongly lateralised too. By contrast, in families were neither parent was strongly lateralised none of the children were strongly lateralised. Although numbers were small, this study elucidates significant familial segregation for strength of functional language lateralisation.

Genetic control of functional language lateralisation seems to depend on handedness. Springer and Searleman (1978) assessed 88 twin pairs and unrelated singletons applying a verbal dichotic listening test. They found similar language lateralisation in right-handed twin pairs, compared to right-handed singletons. However, discordant twin pairs did not show the same heritability pattern. Significant intra-pair correlations were found only in MZ twins of equal handedness, compared to insignificant correlations in MZ pairs with discordant handedness. The large intra-pair differences in MZ twins of unequal handedness were greater than intra-pair differences in DZ twins of unequal handedness. Sommer, et al. (2002) made a similar comparison in their study with 25 MZ twin pairs assessed for language lateralisation using fMRI. The intra-pair correlation for lateralisation index was significantly larger in pairs who were both right-handed, compared to discordant twin pairs. It seems that MZ twins do resemble each other for language lateralisation, at least when twins are concordant for handedness. Other studies also showed that the genetic influence on cerebral asymmetry in right-handed twin pairs is diminished when one of the twins is left-handed (Geschwind, et al., 2002). However, this finding is not consistent across all studies. Jäncke and Steinmetz (1994) also applied verbal dichotic listening to 20 MZ twin pairs and their singleton siblings. Intra-pair correlation for lateralisation of all 20 MZ pairs was not significant. Even MZ twins concordant for handedness had lower language lateralisation concordance than their singleton siblings. At the moment it is not clear whether the same genetic mechanism is driving language functional lateralisation and handedness, but this possible genetic dependency should be addressed by further studies.

In summary, findings are inconsistent, but they point to the obvious conclusion, that functional language lateralisation is determined by an interplay between genes and environment (Bryden, 1975; Anneken, et al., 2004) with handedness playing an uncertain but seemingly important role (Springer and Searleman, 1978; Sommer, et al., 2004). However, to what extent is heredity crucial for functional asymmetry is still debatable, and needs further investigation. The next section will address whether the same is true for the heritability of language anatomical asymmetry.

Heritability of variation in anatomical language lateralisation

One way to study anatomical lateralisation of language is to measure the asymmetry of the planum temporale, observed in foetal brains already by 29-31 weeks of gestation (Wada, et al., 1975). The planum temporale is the upper surface of the posterior temporal lobe, which overlaps partly with Wernicke's area and is thereby involved in language function (Shapleske, et al., 1999). Importantly, it is thought to be larger on the left side of the brain in the majority of subjects providing an indication of cerebral dominance for language (Geschwind and Levitsky, 1968). Using this approach, most studies found insignificant genetic effects on language lateralisation. Steinmetz, et al. (1995) measured asymmetry in 20 MZ twin pairs and observed low intra-pair correlation for planum temporale asymmetry, indicating insignificant genetic and widespread environmental influences. However, this finding might have been influenced by the inclusion of twins of unequal handedness. Eckert, et al. (2002) examined the heritability for planar asymmetry in 12 DZ and 27 MZ male twins who were between 6 and 16 years of age. There was weak but positive evidence for heritability of planar asymmetry. Co-twin similarity for planar asymmetry increased when twins discordant for handedness and birth weight differences of more than 20% were excluded from the analysis. These results suggest that perinatal factors affect the development of planar asymmetry in twins.

In a similar manner, Geschwind, et al. (2002) analysed anatomical asymmetry of the frontal and temporal lobes in 72 MZ and 67 DZ older male twin pairs. Their study supported the notion that cerebral asymmetry is strongly correlated with handedness, since concordances for handedness in twins played a big role in the heritability of language lateralisation. Significant intra-pair correlations were found only in MZ twin pairs who were both right-handed, compared to MZ twin pairs with one left-hander. It was reported that genetic factors contributed twice the influence to hemispheric volume in right-handed twin pairs, suggesting a large decrement in genetic control of cerebral volumes in the non-right-handed twin pairs (Geschwind, et al., 2002). The main result reveals strong lateralised effects of the shared environment (C) over brain volumes. These effects were almost twice as strong in frontal and temporal regions of the left language dominant hemisphere compared to those in the right hemisphere (Geschwind, et al., 2002). Hence, it is open to speculation whether this is also true for the arcuate fasciculus, the tract underlying these brain regions. Nevertheless, it has been previously demonstrated that a large sample is necessary to detect C effects (Posthuma and Boomsma, 2000) and the present study lacks the sufficient power to do so. Conversely, most other studies have observed high genetic effects on the cerebral asymmetry, and strong lateralised effects of genes over the left hemisphere.

It has been suggested that the cerebral hemispheres might experience different genetic influences on cortical morphogenesis, with the language-dominant left cerebral cortex under stronger genetic control in adults (Tramo, et al., 1995) and paediatric twins (Yoon, et al., 2010). These left-lateralised genetic effects on cortical morphogenesis seem to be mostly involved in the language processing system. The only study that examined more specifically the language-related regions using genetic correlation maps, also found significant asymmetry in the heritability of these grey matter areas across hemispheres (Thompson, et al., 2001). Both Wernicke's and Broca's area displayed highly significant heritability on the left but not on the

right hemisphere in adult twins. However, the results should be interpreted with some caution, since the study sample was very small (10 MZ and 10 DZ twin pairs) and lacked statistical power.

There has only been one study to date that examined specifically at the lateralisation indices of the fibre integrity measures for perisylvian pathway using diffusion tensor imaging. In a study done by Jahanshad, et al. (2010) involving 374 adult twins and siblings, voxel-based statistical maps were used to calculate genetic effects on lateralisation measures of the SLF. Three diffusion measures were used: geodesic anisotropy (tGA), fractional anisotropy (FA) and mean diffusivity (MD). The intra-pair correlations for tGA asymmetry were low in MZ twins (0.29), and zero in DZ (0.00), hence pointing to high environmental effects. Further SEM analysis revealed minor genetic influences for SLF fibre asymmetry as measured by tGA (a^2=0.24) and FA (a^2=0.21), while small shared environmental effects were noticed for the asymmetry of MD measures (c^2=0.10). The results indicated that the asymmetry of SLF is mostly influenced by unique environmental effects, with negligible contributions from genes and shared environment.

Studies on the heritability of language asymmetry are inconsistent. Most of them agree that there is a difference in heritability pattern across hemispheres, but the inconsistencies are based around the magnitude of genetic or environmental effects that play a role. However, there are also studies that found no difference in the heritability pattern of the two hemispheres. Wright, et al. (2002) suggests that random or fluctuating asymmetry in bilateral structures is not heritable, and Hulshoff Pol, et al. (2006) found that both the left-and right-hemisphere representations of anatomical brain regions share the extent to which their individual differences in grey and white matter density are genetically determined. Further studies are needed to discern the importance of genetic versus environmental effects on brain asymmetries.

4.1.4.3 Final remarks

The conclusion from these several lines of research points to high genetic effects on language brain regions, but small genetic control on lateralisation of language function and anatomy. To ponder the reasons why there are differences in the heritability of language regions versus language lateralisation we have to more closely examine the context in which these findings arise.

To begin with, we know that asymmetry cannot be influenced exclusively by an individual's genotype, as many identical twins are discordant for handedness (Toga and Thompson, 2003). The intra-pair correlations for language lateralisation in monozygotic twin pairs are low (hardly exceeding that of dizygotic twins). Thus, it is assumed that the environment is the key player - acting through different mechanisms such as pathology (Coren, 1992), learning (Provins, 1997), culture (Collins, 1977), or a combination of variables (Perelle and Ehrman, 2005). However, this assumption may be premature since several other non-environmental factors can affect language lateralisation in twins. For example, the genetic models may involve a random factor, which produces low concordance even in MZ twins; or they can be a result of disruption of embryonic asymmetry development by the twinning process itself, a phenomenon called "mirror-imaging" (Sommer and Kahn, 2009). Second, besides these genetic factors, it is possible that environmental factors are more

prominent in twins. For example, it is known that twin birth is more difficult than singleton birth. Twins are at increased risk of low birth weight, preterm birth, prolonged birth, prolonged labour, aberrant foetal position at birth, bleeding complications, and asphyxia (Norwitz, et al., 2005). If we reflect on the possibility that environmental influences play a bigger role in twins than singletons then the crucial question is whether these lateralisation studies overestimate the importance of environmental factors in twins. Taking all these reasons into account, some authors argue against extrapolating the findings on heritability of lateralisation in twins to predict heritability in singletons. The classic twin design may therefore not be the ideal method to test heredity of language lateralisation (Sommer and Kahn, 2009).

Within the studies that examined the inheritance of language-related brain regions there are inconsistencies as well. Discrepancies in MRI findings can be explained by several factors. First, the age difference of twin samples may be important. The interplay between genes and environment is a dynamic process, and hence the influences of genes and environment most likely change over one's lifetime. Potential contributors to changing heritability are age-dependent gene expression (Plomin, et al., 1997; Sun, et al., 2005) or gene-environment correlation, which occurs when the same genes affect both a trait and relevant features of the environment (Lenroot, et al., 2009). Regions associated with complex cognitive processes such as language were suggested to be more heritable in adolescents than children (Lenroot, et al., 2009). This is consistent with previous studies noting that cognitive abilities such as prosocial behaviour, IQ and g become increasingly heritable with maturity (Plomin, et al., 1997). Nevertheless, a recent DTI study demonstrated that genetic influences on FA are greater in adolescence than adulthood (Chiang, et al., 2010). This observation might be understandable if we recall that many highly heritable neuropsychiatric disorders, involving language and social cognition, have their peak age of onset during adolescence (Wallace, 2006). It is therefore, perhaps, unsurprising that the genetic control is higher during that time. Hence, the results of the present study will not be generalisable, but will be specific to the age-range tested (adulthood).

A second reason for discrepancies in MRI findings is the different methodology for brain volumetric and morphometric measures such as grey matter density (Thompson, et al., 2001; Hulshoff Pol, et al., 2006), cortical gyral pattern (Hulshoff Pol, et al., 2006; Lenroot, et al., 2009), cortical thickness (Joshi, et al., 2011), fractional anisotropy (Chiang, et al., 2009) and so on.

Fig 4.2.1 Descriptive explanation of the methodological steps taken in the present twin study

4.2 Methods

Subjects

Eighty-six twin subjects (26 monozygotic (MZ) and 17 dizygotic (DZ) pairs) were recruited from a volunteer twin register at the Institute of Psychiatry, London, England and by national media advertisements. Exclusion criteria applied were age younger than 18 years, a history of psychiatric and neurological disorder or of systemic illness with known neurological complications, a history of significant head injury associated with loss of consciousness for more than one minute, and current harmful substance use or dependence (defined as within the last 12 months). The age range was 21-56 years for MZ and 20-62 years for DZ pairs. The U.K. Multicenter Research Ethics Committee has granted the approval, and all of the subjects gave written informed consent before participating. Descriptive statistics is shown in Fig 4.2.1 and Table 4.2.1.

	MZ	DZ
Number of Subjects	52	34
Mean Age	35.54	42.53
Age Range	21-56	20-62
Females	34	26
Males	18	8

Table 4.2.1 Demographics of the twin data used in the study

DT-MRI acquisition

Data was acquired on a GE Signa 1.5-T LX MRI system (General Electric, Milwaukee, WI) with 40-mT/m gradients, using an acquisition sequence fully optimized for DT-MRI of white matter, providing isotropic resolution (2.4 x 2.4 x 2.4 mm) and coverage of the whole head. This acquisition was gated to the cardiac cycle using a peripheral gating device placed on the subjects' forefingers. TE= 104.5ms, there were 60 slices, 32 uniformly distributed directions, 6 b0 images, with a b-value= 1300 s/mm^{-2}.

DT-MRI processing

Following correction for the image distortions introduced by the application of the diffusion encoding gradients using in-house software (Jones, 2002), the diffusion tensor was determined in each voxel following the method of Basser (1994). Four different quantitative indices were estimated in each voxel (number of streamlines, volume, fractional anisotropy (FA) and mean diffusivity (MD)). To ensure that the observer was blind to zygosity during virtual dissection of the language pathways and to provide protection against subjective bias, DT-MRI subjects were blinded and randomised, thus withholding the information on the twin pairs. FA treshold was FA less than 0.2, and angle threshold was 35 degrees.

Tractography Algorithm and ROI delineation

Tractography was performed using in house built software and was based on the procedure originally described by Basser (2000). Firstly, a continuous description of the diffusion tensor field was obtained using a B-spline fitting on the elements of the tensor from each voxel (Basser, 2004). This procedure allows rapid evaluation of the DT at any arbitrary location within the image. The regions of interest were selected, and the voxel inside these regions considered as the starting point of the tractography (“seed points”). For each

seed point we propagated the streamline following the directions of the principal eigenvector. The track was propagated by 0.5 mm step along this direction. The diffusion tensor was then determined at this new location and the orientation of its principal eigenvector estimated. The procedure was repeated iteratively. A pathway was tracked until the fractional anisotropy of the tensor was below a fixed arbitrary threshold (0.2) or the curvature was less than 30 degrees. The procedure was then repeated by tracking in the opposite direction, to reconstruct the whole tract passing through the seed-point.

ROI delineation method

A two regions of interest (ROI) approach described in Catani et al., (2005) has been used in this study to dissect the three segments of the perisylvian pathways. Please refer to Methods section of Chapter 2, to read more about this procedure.

Genetic Model Fitting

Assumptions of the Twin Design

Distributional Assumption

Normal distribution was found for all diffusion measures (number of streamlines, volume, fractional anisotropy and mean diffusivity). This was tested using 1-Sample KS test in SPSS.

Homogeneity of variances and means

There were no significant differences in the means and variances of diffusion measures within and across zygosity (MZ and DZ), as tested by Levene's test (for variances) and paired t-test (for means). Data is included in the Appendix A, Table 4.2.1.

Equal Environment

In our sample, there is no evidence of increased MZ environmentally influencing MZ co twin correlations (which would inflate genetic contributions, and underestimate common environment effects). Therefore, equal environment assumption is met.

Power and Sample Size

An important limitation in using twin analysis in neuroimaging is the necessity for large sample sizes in order to have confidence in the results. Samples in the hundreds offer very little statistical power, so only full model (i.e. ACE model, and not sub-nested models like AE, CE etc.) results was considered. For that reason we employ full ACE model results.

Preparation of data prior to model fitting

For quantitative genetic model-fitting, the scores of age, and sex were regressed out in SPSS and the standardized residuals used. This was done for several reasons. First, the mean age between MZ and DZ twins differed significantly. Second, gender differences and age-related changes in diffusion measures influence tracking results (Catani et al, 2005; Lebel et al, 2008, 2010; Lebel and Beauieu, 2009). Third, significant modulatory effects of age and sex on the heritability of white matter as measured by FA have previously been reported (Chiang, et al., 2010). And last, it is believed that members of same-sex twin pairs

are more likely to be similar to one another than members of opposite-sex twin pairs. Therefore, because MZ twins are necessarily the same sex, whereas DZ twins may be opposite sex, if one does not exclude opposite-sex DZ twin pairs, heritability estimates may be inflated (Stromswold, 2001). Although in this study I only used the same-sex DZ twin pairs, I used sex as a covariate in the later analysis to remove the possible different effects of male versus female twin pairs on the heritability results.

Handedness was added as additional covariate (measured with the Edinburgh Handedness Inventory (Oldfield, 1971), since it has been reported that handedness has effects on language lateralisation (Geschwind, et al., 2002; Vernooij, 2007; Springer, 1999; Pujol, 1999).

Estimation of genetic and non-genetic contributions

The comparison of interclass correlation coefficients (ICC) in MZ and DZ twins provided an initial, descriptive statistic of the presence of genetic effects and were computed for the three segments of the arcuate fasciculus using quantitative measures of FA, MD, Number of Streamlines, Volume and Laterality Index (as calculated by procedure explained in Methods Chapter 2) using SPSS. Causative influences of variability were divided into additive genetic (A), shared environment (experiences that make children growing up in the same family similar, C), and unique environment (environmental influences that contribute to differences between family members (E)) (Plomin, 2001). Non-additive genetic influences (D) replace C in ADE model. Formal estimation of the genetic and environmental components of variance was assessed in two ways: with preliminary analysis using the Falconer method and confirmed with more advanced structural equation modelling (SEM).

Falconer analysis

For all three segments of the arcuate fasciculus, we computed Falconer's heritability estimate (h^2) (Falconer and Mackay, 1995), by calculating intra-class correlations ICCs (Scout and Fleiss, 1979) which provide descriptive evidence of genetic influences on diffusion measures. ICCs, as the general measures of resemblance, help estimate the proportion of variance due to additive genetic (A), dominant genetic effects (D), common environment (C) and unique environment (E) effects. The formulae for calculating variances are shown below, with ICC written as r.

A (h^2) = $2(r_{MZ} - r_{DZ})$ assuming $D \cong 0$;

$C = 2r_{DZ} - r_{MZ}$;

$D = 2r_{MZ} - 4r_{DZ}$;

$E = 1r_{MZ}$:

Univariate Model Fitting

Model fitting was carried out using raw data and the structural equation modelling program OpenMx to provide parameter estimates and confidence intervals (Neale et al, 2003). Maximum likelihood estimates of a^2, c^2, and e^2 were obtained, 95% confidence intervals calculated and a series of nested models compared. Model selection among hierarchically nested models was based on likelihood-ratio tests using (chi-square) test (LRT), and the Akaike information criterion to compare untested models. The LRT uses the log likelihood statistic -2LL with associated p values and is used to select the model with the best fit given the number of degrees of freedom (df). A full ACE model was compared with an AE model (excluding common environmental factors), CE model (excluding additive genetic effects), and an E model (excluding all familial resemblance). Statistical power for univariate twin analyses can become an issue in cases where effect sizes, sample sizes, or trait prevalence are low (Neale et al., 1994). Also, reliance on the use of global fit indices such as Akaike's information criterion to select among nested models can be problematic (Sullivan and Eaves, 2002). Therefore only full model estimates are reported in the final results (full ACE model). Furthermore, even if the role of the shared environment (C) is not statistically significant, calculating heritability estimates from an AE model will upwardly bias the estimates. Figure 4.2.2 outlines the univariate ACE model used in this study. Latent factors A (additive genetic), C (shared environment) and E (non-shared environment) are shown as acting on diffusion measure (FA) of arcuate fasciculus, via paths a, c, and e.

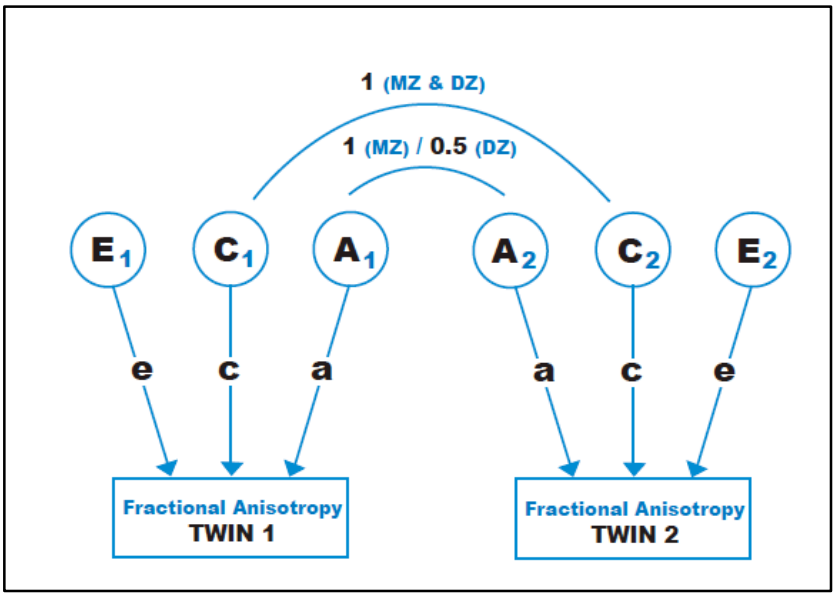

Fig 4.2.2 Univariate ACE model of Fractional Anisotropy measure for one twin pair. It is assumed that there are additive genetic (A), shared environment (C), and unique environment (E) factors acting on the measured variable. These are assumed to be the same for each member of a twin pair: a, c and e provide estimates of the variance due to additive genetic, shared environment, and unique environment factors. Genetic correlation between twins in a pair is 1 in MZ pairs and 0.5 in DZ pairs. Shared environment correlation is assumed to be 1 for both MZ and DZ.

4.3 Results

DTI dissections

Tractographic dissection of the three segments of the arcuate fasciculus were performed for both MZ and DZ twins, and these are shown in Fig 4.3.1.

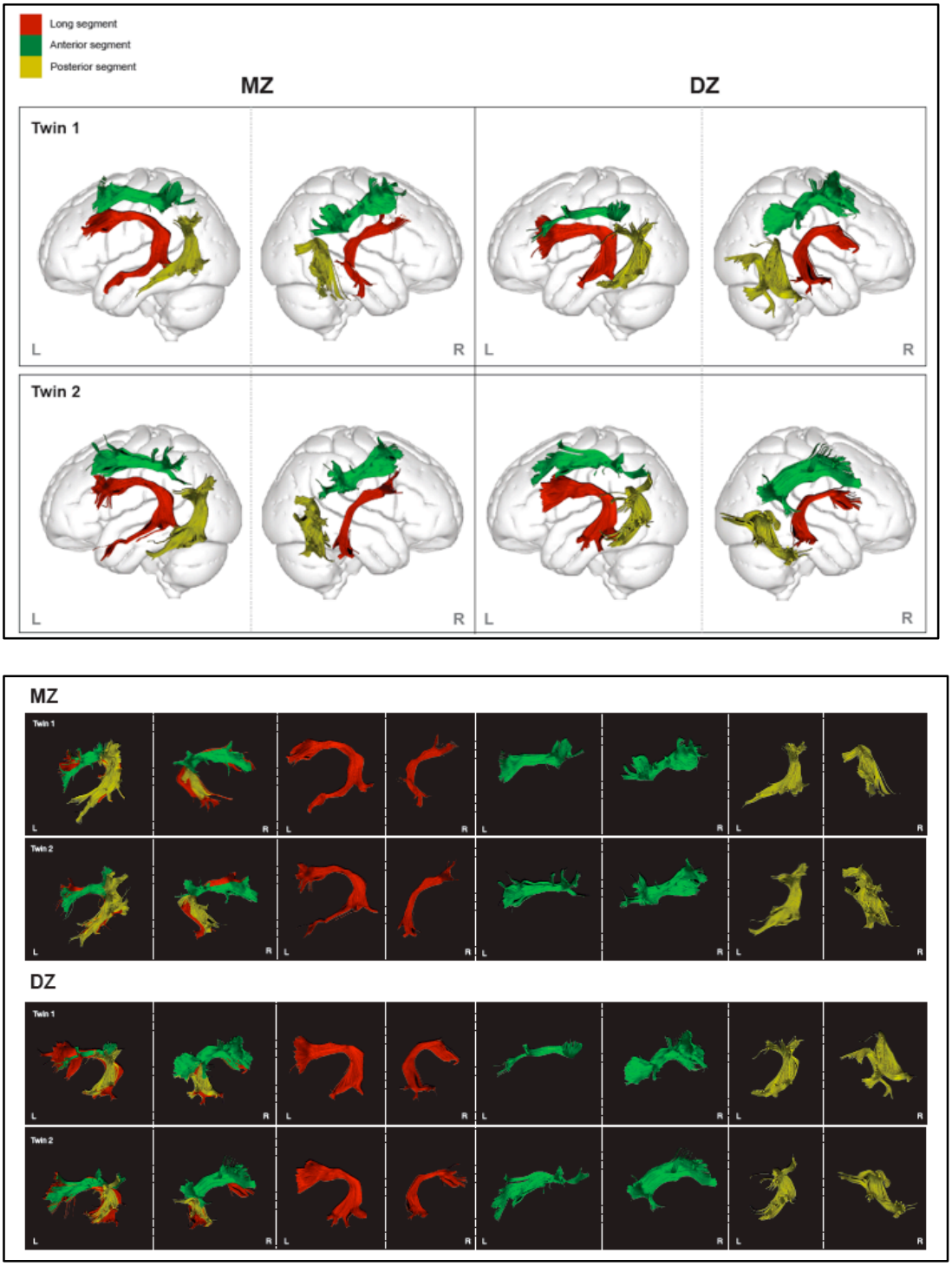

Fig 4.3.1 Descriptive example of DTI dissections of the three segments of the arcuate fasciculus in the left and the right hemisphere for one representative pair of monozygotic (MZ) and dizygotic (DZ) twins; the long direct segment is shown in red, the anterior indirect segment is shown in green, and the posterior indirect segment is shown in yellow.

Intra-class correlation coefficients (ICC) results

I created a so-called Correlation Bubble Diagram, in order to visualize better the differences between MZ and DZ twins in intra-class correlations of different diffusion measures of the three segments across hemispheres (see Figure 4.3.2 below). Each Correlation Bubble ranges from values 0 (no correlation) to 1 (perfect correlation), with MZ and DZ Bubbles superimposed on top of each other for visual guidance of correlation differences. As expected from the twin studies of brain structure (Thompson et al, 2001) MZ twins showed higher intra-class correlation coefficients than DZ for all diffusion measures, suggesting considerable influence of familial effects on all DTI measures (genetic and shared environment, A+C).

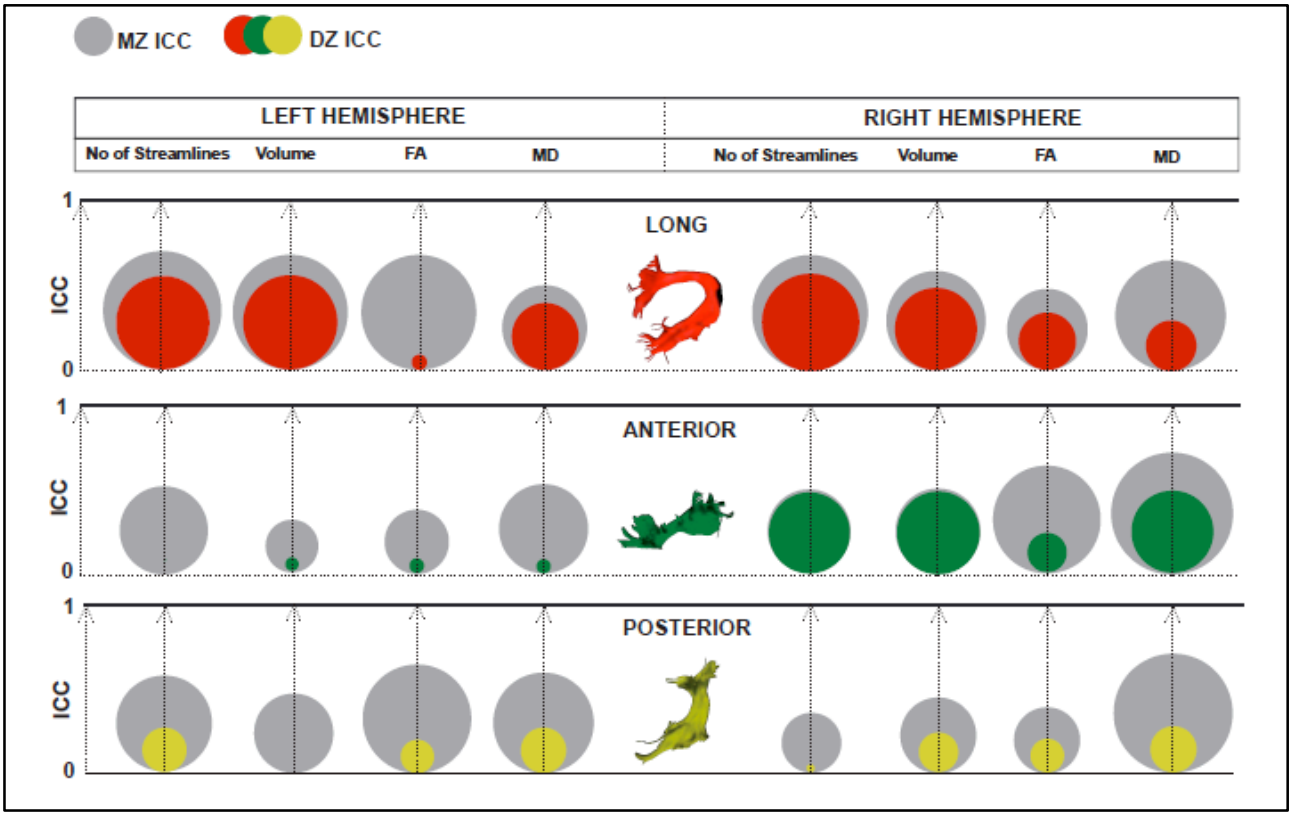

Fig 4.3.2 Bubble diagram of intra-class correlation coefficients for diffusion measures of MZ (in grey) and DZ (in colours) twins of the long, anterior and posterior segment.

SEM analysis: Univariate Twin Modelling

To separate genetic and environmental factors acting on the perisylvian language network I performed SEM analysis using OpenMx (for SEM results see Fig 4.3.3). As the sample size was too small for the standards of quantitative genetics, I had low power to test the hypotheses a=0 or c=0. Therefore, although nested models were examined (CE, AE, E) I derived estimates for the a^2, c^2, and e^2 parameters, and their 95% confidence intervals, from the ACE model. Due to the moderate-sized twin study, the width of confidence intervals for heritability estimates is sometimes considerable (See Table 4.3.1, Appendix A). Where A and C are non-significant individually, but significant together, they are reported together as familial effects (A+C), as previously reported by Wright, et al. (2002).

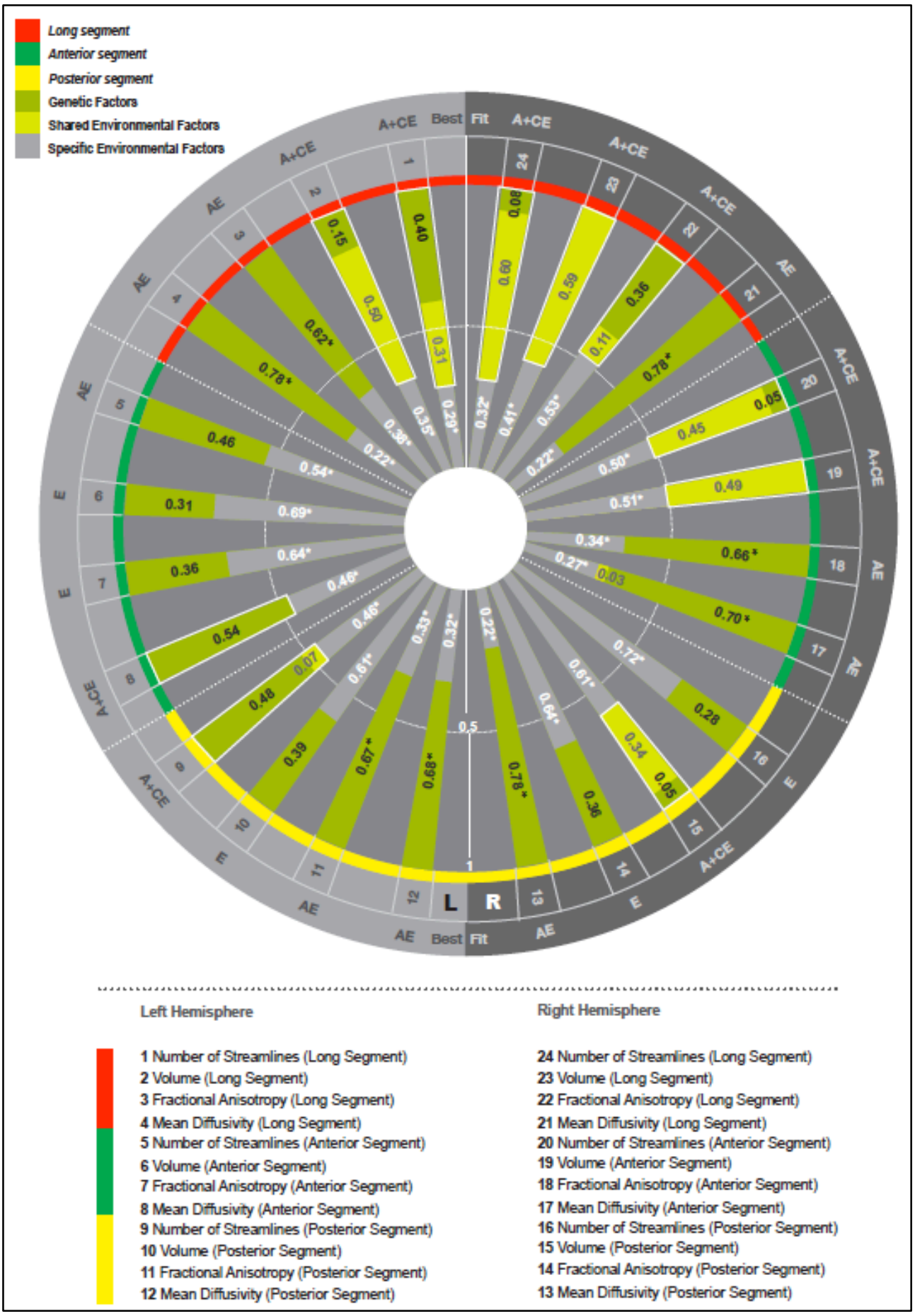

Fig 4.3.3 Results of the SEM analysis for the three segments of the arcuate fasciculus in the left and right hemisphere.

a) Asymmetry of heritability estimates - hemispheric differences

SEM analysis showed that overall genetic control was similar across hemispheres, with the exception of FA heritability, an index sensitive to the degree of myelination, fibre diameter, axonal density and coherence (Beaulieu, 2002). FA heritability pattern was highly sensitive to anatomical lateralisation, with significantly higher genetic control in the dominant hemisphere (left hemisphere for the long segment, right hemisphere for the anterior segment) (See Fig 4.3.4 bellow).

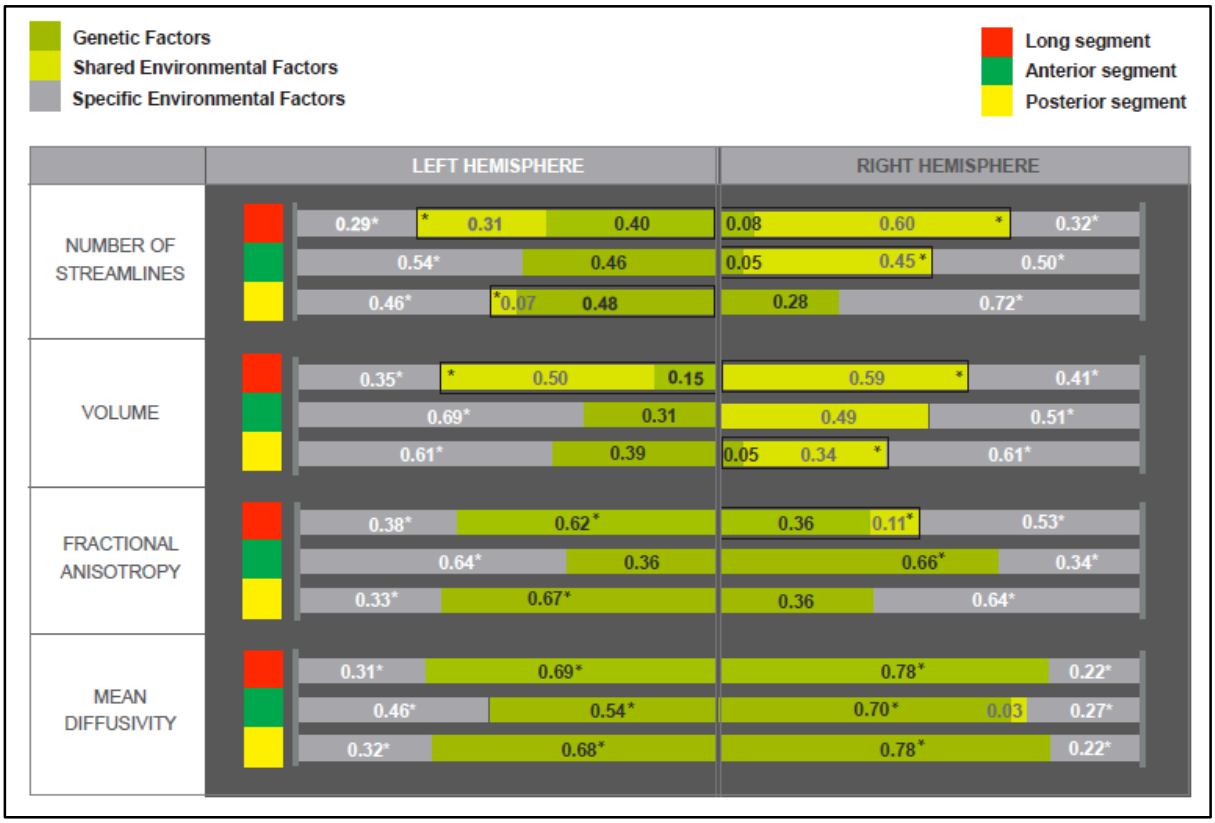

Fig 4.3.4 Genetic (A), shared environmental (C) and specific environmental (E) effects on the three segments of the left and right hemisphere; asterisks (*) represents significant confidence intervals; where A and C are non-significant individually, but significant together (familial effects A+C) a box is drawn around both A and C values.

b) Heritability differences of DTI-extracted parameters

DTI-extracted parameters showed different degrees of heritability. In general, the measures of fibre integrity (FA and MD) showed higher genetic control compared to volumetric measures (number of streamlines and volume) (please refer to Fig 4.3.4). The highest genetic control was observed for fibre integrity measured by MD, where genetic effects achieved statistical significance across all the tracts and hemispheres. 54-78% of the inter-subject variability in MD across hemispheres was explained by genetic factors. Similarly, high genetic factors, which reached statistical significance, were found for FA in the dominant hemisphere (62-66%). However, genetic factors acting on volumetric measures of the three segments across hemispheres did not reach statistical significance. In all three segments of the arcuate fasciculus volumetric measures were under higher environmental control, with the exception of the long

segment that matures and lateralises very early in life, and shows significant familial effects (A+C). However, due to the wide confidence intervals (see Table 4.3.1, Appendix A) I was not able to separate these familial effects and determine the exact size of genetic versus common environmental factors.

c) Heritability differences of the three perisylvian language segments

Heritability estimates of the long segment of the arcuate fasciculus showed overall the highest degree of genetic (FA and MD) and familial (number of streamlines and volume) effects, which acted to the same extent in both hemispheres, compared to the anterior and posterior segments (see Fig. 4.3.5) . Variability in DTI measures of the anterior and posterior segments were more affected by the unique environmental factors (except MD). The posterior segment on the right showed the highest degree of unique environmental factors (except on MD measures).

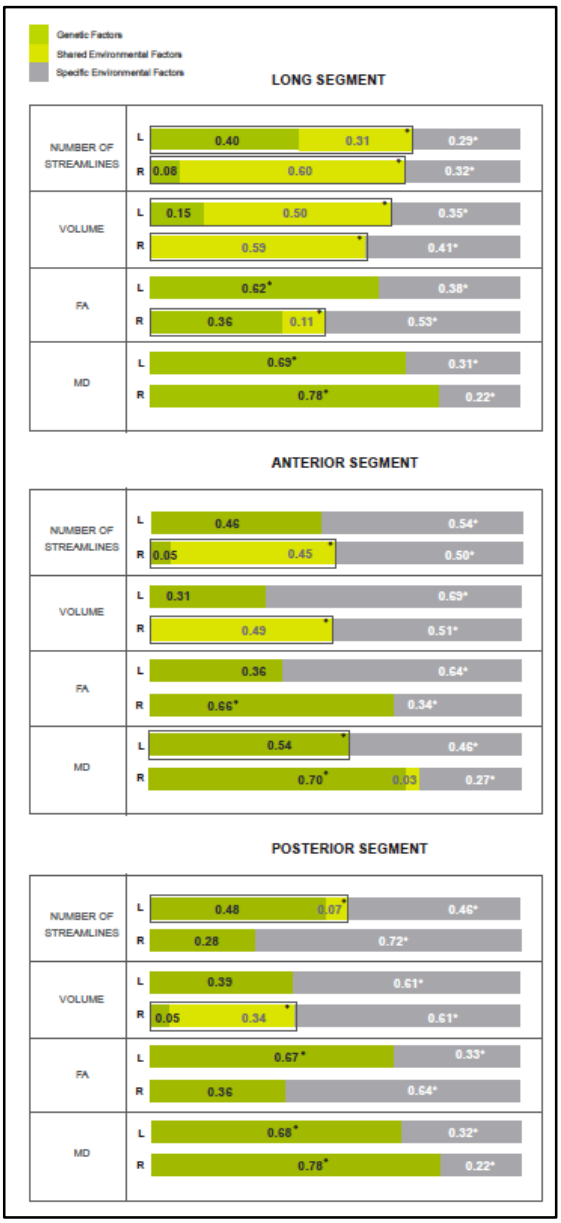

Fig 4.3.5 A, C and E effects on the variability of diffusion measures of the three segments, where A+C+E=1; asterisks (*) represents significant confidence intervals; where A and C are non-significant individually, but significant together (familial effects A+C) a box is drawn around both A and C values.

d) Heritability of the lateralisation patterns

SEM analysis revealed significant genetic influences on the long segment fibre asymmetry as measured by FA (a^2=0.63) and MD (a^2=0.61), while significant familial effects (A+C) were noticed for the asymmetry of the number of streamlines (a^2=0.39, c^2=0.23) and volume (a^2=0.41, c^2=0.08) measures (see Fig 4.3.6). In contrast, the results indicated that the asymmetry of the anterior and posterior segments is mostly influenced by unique environmental effects, with an E model yielding the best fit for all the measures used.

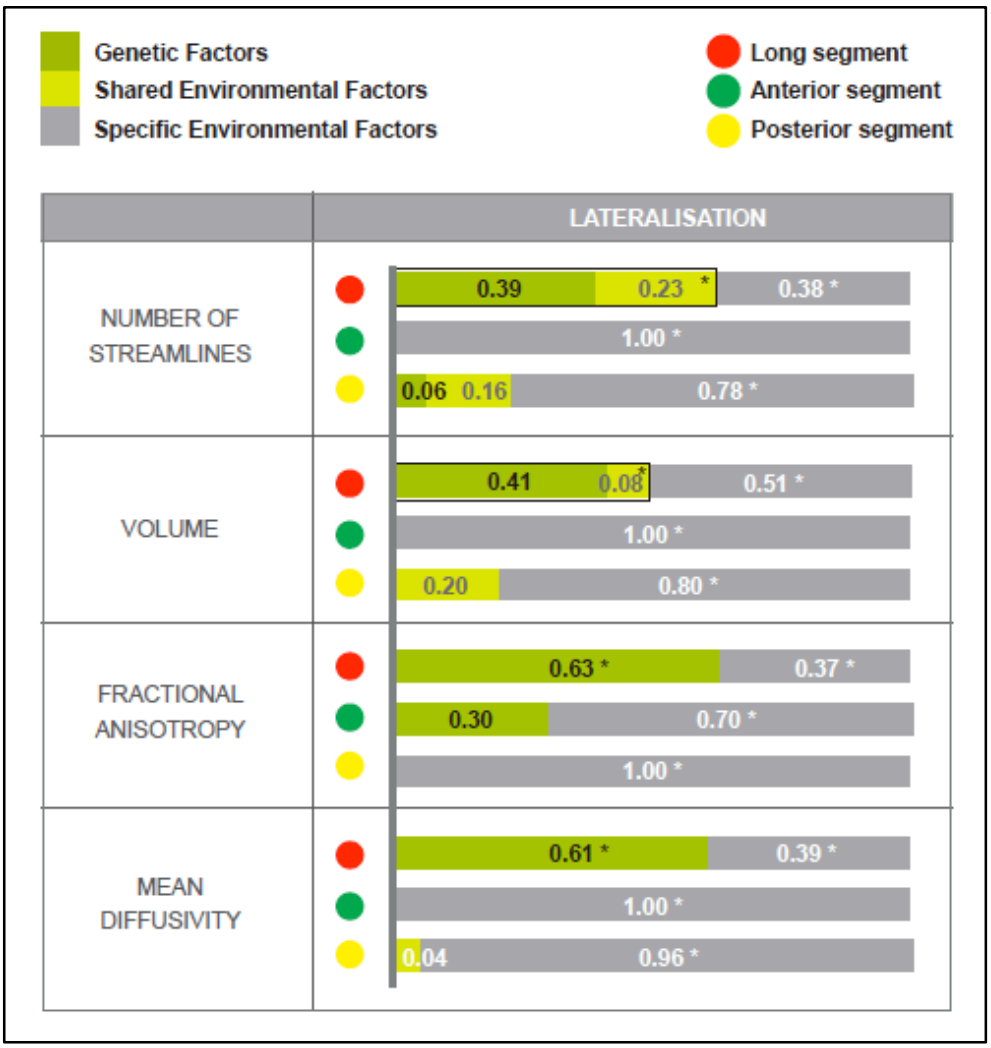

Fig 4.3.6 A, C and E effects on the variability of the lateralisation of the three segments, where A+C+E=1; asterisks (*) represents significant confidence intervals; where A and C are non significant individually, but significant together (familial effects A+C) a box is drawn around both A and C values.

4.4 Discussion

This is the first time that the heritability of the perisylvian white matter network underlying language has been analysed comprehensively in a classical twin study design. Using the methods of quantitative genetics and brain imaging I measured the degree of genetic control over the variability in the three segments of the arcuate fasciculus, through analysis of white matter integrity (MD and FA) and white matter volume (number of streamlines, volume) in twins.

Several findings have arisen from this study. First, different inheritance patterns were observed for different segments of the arcuate fasciculus. The highest genetic and familial control in adulthood was observed for the variability of the long segment that matures and lateralises very early in life (see Chapter 2), with these effects present to an equal extent in both hemispheres. In contrast, individual differences in the anatomy of the anterior and posterior segments were mostly affected by the unique environmental factors (except for MD measures). This is contrast to prior studies which reported that frontal lobe white matter is more environmentally driven, as compared to posterior brain regions (Brun, et al., 2009). Although the wide confidence intervals merit cautious interpretation, it is noteworthy that the right posterior segment is the language structure with the lowest additive genetic effects. We can speculate that high environmental control of the right posterior segment is related to it having a more protracted maturational course, and being implicated in higher cognitive functions that continue to develop throughout adulthood, such as theory of mind, abstract thinking, language pragmatics and so on. Our results are in line with the findings of higher degree of environmental contribution for those brain structures that mature later in cerebral development (Brun, et al., 2008; Lohmann, et al., 1999).

Regarding the heritability of language asymmetry, the findings from prior studies are inconsistent. Most agree that there is a difference in heritability pattern across hemispheres, but there are inconsistencies in the magnitude of genetic versus environmental effects. In this study SEM analysis revealed significant genetic control of the long segment fibre asymmetry as measured by FA (a^2=0.63) and MD (a^2=0.61), while significant familial effects (A+C) were noticed for asymmetry in the number of streamlines (a^2=0.39, c^2=0.23) and volume (a^2=0.41, c^2=0.08). This is contrary to a recent finding by Jahanshad, et al. (2010) who reported only minor genetic influences for FA fibre asymmetry of the long segment. The results of my study suggest that the variability in the lateralisation pattern of the long segment, that arises very early in life favouring the left hemisphere, is mostly influenced by genetic or familial effects, with only minor contributions from unique environmental factors. However, individual differences in the asymmetry of the anterior and posterior segments, that tend to be more dynamic in nature (see Chapter 2), are mostly influenced by unique environmental effects. This is in line with the suggestion by Wright, et al. (2002) that random or fluctuating asymmetry in bilateral structures is not heritable. What implications these results have on the maturation study (Chapter 2) will be discussed later (Chapter 7).

Different heritability patterns were observed for different DTI measures. This indicates that, in adulthood, genetic factors act to a different degree on different aspects of white matter anatomy. DTI-extracted parameters of white matter integrity, highly sensitive to the degree of axonal myelination, average axonal diameter and membrane integrity (FA and MD) showed higher genetic control compared to volumetric measures of white matter (number of streamlines, volume). In all three segments of the arcuate fasciculus volumetric measures were under higher environmental control, with the exception of the long segment that that matures and lateralises very early in life, and shows significant familial effects (A+C). High environmental effects on the volume measures are not surprising given that the brain volume continues to increase after birth, showing progressive and regressive changes during life, partly driven by experience (Fields, 2008) My results indicate that in adulthood modifications in the number of brain connections are driven mainly by familial effects on the long segment, in contrast specific environmental factors impact on the anterior and posterior segment. This suggests that dynamic changes in perisylvian white matter volume are driven by stimulation from the environment (specific or shared). My study also lends support to the notion that experience changes white matter (Fields, 2008; Toga et al., 2006) and can greatly increase the number of white matter connections underlying language processing. In contrast, white matter microstructure of perisylvian language pathways encompassing axonal membrane integrity, degree of myelination, orientation of fibres and fibre diameter is mostly predetermined by our genetic makeup. These findings may explain why DTI studies of highly heritable neurodevelopmental disorders, for example autism, consistently found abnormalities of white matter integrity (FA and MD measures) but not white matter volume of the perisylvian language pathways (Ameis, et al., 2011; Fletcher, et al., 2010) linked to functional language deficits in autism (Levy, et al., 2012). However, genes and environment are not independent of each other, and genetic factors can drive the exposure to certain environmental settings and relevant experiences.

The highest heritability among DTI parameters I observed was for MD of water diffusion, where 54-78% of the inter-subject variability across hemispheres was explained by genetic factors. Similarly, high genetic factors were observed for FA, an index sensitive to the degree of myelination, fibre diameter, axonal membrane integrity (Beaulieu, 2002). Previous DTI studies observed a significant contribution of genes to FA measures in bilateral fronto-temporal segment of the arcuate fasciculus (long segment) in adults (Chiang, et al., 2009; Kochunov, et al., 2010). However, my results suggest that FA inheritance is highly sensitive to anatomical lateralisation, with significantly higher genetic control in the dominant hemisphere (left hemisphere for the long segment, and right hemisphere for the anterior segment). Other DTI findings found no such asymmetry, however might be due to different methods used, such as voxelwise analysis (Chiang, et al., 2009) and tract-based spatial statistics (Kochunov, et al., 2010) versus tractography used in this study. Reports of lateralised genetic effects on brain structure are not uncommon, but are inconsistent. There are findings of the left hemisphere being under greater genetic (Joshi, et al., 2011; Lohmann, et al., 1999; Pell, et al., 2009; Tramo, et al., 1995; Thompson, et al., 2001; Yoon, et al., 2010) or environmental control (Geschwind, et al., 2002; Carmelli, et al., 2002). My study showed that in adulthood the inheritance of FA exhibits an asymmetrical hemispheric pattern reflecting the anatomical asymmetry of the perisylvian language network. However, the magnitude of genetic effects varies with age (Lenroot, et al., 2009), and so does FA, which increases during development due to progressive myelination. In childhood no significant

effects were found for FA of the arcuate fasciculus (long segment) (Brouwer, et al., 2010). Thus, it is likely that the heritability of FA increases from childhood to adulthood, in line with the reports that complex cognitive processes such as language become increasingly heritable with maturity (Lenroot, et al., 2009), with the increases prevalent in the dominant hemisphere.

However, in all three segments of the arcuate fasciculus volumetric measures were under higher specific environmental control, with the exception of the long segment that matures and lateralises very early in life, and shows significant familial effects (A+C). High genetic effects acting on the white matter density of the long segment (SLF) were found previously in a study by Peper et al. (2009), with heritability estimates ranging from 76 to 91% in paediatric populations. Although their study used a voxel based approach, and their results need to be interpreted with some caution since no actual fibre-bundles could be traced on the T1-weighted brain images, taken together our results indicate that throughout life genetic/familial effects are significant for the long segment. In contrast, volumetric measures of the anterior and posterior segment are more environmentally driven. This is not surprising given that we know brain volume continues to increase after birth, and shows progressive and regressive changes during life. My results indicate that in adulthood modifications in the number of brain connections are driven mainly by familial effects for the long segment, and specific environmental factors for the anterior and posterior segment - meaning that dynamic changes in perisylvian white matter volumes respond significantly to stimulation from the environment (specific or shared). However, genes and environment are not independent of each other, and genetic factors drive the exposure to certain environmental settings and relevant experiences.

There are several limitations arising in my study design. First, there are technical limitations of DT-MRI tractography such as inability to solve crossing or kissing of fibres leading to possible presence of false positives and false negatives (Catani and Dell'Acqua, 2011). However, all three segments of the arcuate fasciculus were visually inspected to ensure that they conformed to known anatomical trajectories. Further, low MRI voxel resolution lacks the ability to characterise cellular mechanisms and thus we were unable to distinguish directly how heritability affects individual cellular components. Second, my results need to be interpreted in the context of the limitations to the classical twin model. These include unequal environments between MZ versus DZ twins - however, research shows that equal environment assumption is generally valid (Plomin, 2001); ascertainment bias; problems with significant gene-environment correlations and interactions; lack of follow-up of the phenotypes over time, and environmental noise (Boomsma et al, 2002). Also, there is a question of whether the results of twin studies are applicable to non-twin populations. They might be regarded applicable only to the extent that twin and singleton brains are alike. Although twins are more likely than singletons to experience adverse prenatal and perinatal events that may affect brain development (Norwitz, et al., 2005), studies showed that in general there are no significant differences in the brain structures of healthy paediatric (Ordaz, et al., 2010) and adult subjects (Hulshoff Pol et al., 2002). Also, twins are slower in language development than singletons, although this delay diminishes during childhood (Rutter & Redshaw, 1991). My study was further limited by sample size, that is small for the standards of quantitative genetics leading to the confidence intervals of additive genetic (A), common environment (C), and unique environment (E) to be wide. Results of power studies show that at least 200 pairs are needed for

obtaining a reasonable estimate of the degree of genetic influence on a highly heritable trait (Rijsdijk and Sham, 2002). A large sample is necessary to detect C effects (Posthuma and Boomsma, 2000) and the present study probably lacks sufficient power to do so. However, this lack of sufficient power was helped by reporting C and A in combination (when they are statistically significant together), and expressing them as 'familial effects' (Wright, et al., 2002). Importantly, it needs to be taken into account that correlation analyses depend on the reliability of the variables used. There is limited information on the reliability of tractography measurements (see Chapter 1.2.2) but there are some indications that the arcuate fasciculus is one of the structures that exhibits the most reliable DTI measurements (Danielian et al., 2010). However, this sheds some doubt on the findings of different inheritance patterns of different tractography measures, which might instead reflect differences in individual reliability (e.g. volumetric measures being less reliable than diffusion measures would lead to lower intra-class correlation coefficients, and hence lower genetic effects).

In conclusion, converging diffusion imaging and genetic data are beginning to elucidate the influences of genes and environment on specific aspects of perisylvian language anatomy. In general, knowing the degree of genetic control on individual features of perisylvian white matter is important since abnormalities of the constituents of the white matter substance have been described in various psychiatric conditions and has vital implications for disorders that manifest with language pathology. Results from this study lend support to the view that different aspects of white matter anatomy are under different hereditary mechanisms: higher genetic control of white matter integrity as compared to volume. DTI-derived features that are more heritable than others (FA and MD), provide biological markers for inherited traits and may serve as targets for linkage and association studies. On the other hand, knowing that volumetric measures are prone to environmental modulations has implications for environmentally-based therapies. Our study further highlights the relevance of fronto-temporal perisylvian pathway (long segment), which is under the greatest genetic (white matter integrity) and familial (volume) control, for the future genetic linkage and association studies in aiding their hunt for genes influencing language-related brain structure and function. Further research though is needed, to replicate and overcome the limitations of the present study, and aid in deciphering the inheritance puzzle of the perisylvian white matter anatomy.

Chapter 5

Introduction to Autism Spectrum Disorders

5.1 Introduction

In my previous chapters I explored how perisylvian language pathways behave in the healthy population, without reference to different neuropathological conditions. In contrast, the third tractography study will explore the anatomy of the perisylvian network in autism spectrum disorder (ASD, including high functioning autism and Asperger's syndrome). Although ASD is not primarily a language disorder, deficits in communication represent an important diagnostic feature, along with problems in social interaction and repetitive and/or stereotyped behaviours. Hence, this neurodevelopmental disorder offers the possibility of correlating white matter abnormalities along the segments of the perisylvian pathways with clinical and neuropsychological assessment of language performance. In order to provide background information to the following autism study, this chapter will introduce autism spectrum disorders and discuss what language deficits have been reported in this disorder up to now.

Autism spectrum disorders (ASDs) are complex neurodevelopmental disorders characterised by a triad of impairments in reciprocal social interaction, verbal and/or non-verbal communication, and repetitive and stereotyped behaviour (DSM-IV, APA 1994). ASD is used as an umbrella term to refer to different autistic subtypes sharing some of these core features and includes: autism, Asperger syndrome, Rett syndrome, childhood disintegrative disorder, and pervasive developmental disorder-not otherwise specified (PDD-NOS). Although my sample satisfied clinical research criteria for childhood autism at the time of recruitment I use the term ASD throughout the PhD thesis to acknowledge the range of participants used and the heterogeneity of the disorder. Also, this decision reflects the future change in diagnostic criteria within DSM-V, which is due in 2013, whereby all the previously related subgroups (Asperger syndrome, autism, and PDD-NOS) will be combined into one diagnosis of autism spectrum disorder. Hence, diagnosis of autism will change from categorical to dimensional, and I will relate to this later in Chapter 6 when discussing the autism sample in my study.

History

The concept of autism is fairly recent. It was first introduced by the pioneering work of Leo Kanner (John Hopkins University) on what he called 'early infantile autism' characterised by severe impairments in social interaction, communication and intense resistance to change. Kanner published his results in 1943, based on eleven children, nine boys and two girls that he thought were suffering from this condition. Unaware of Kanner's publication, in a war-struck Europe, Hans Asperger, an Austrian paediatrician, published his paper only one year later, describing children unable to form social relationships with their peers. Asperger used the term 'autistic psychopathy', which might seem strangely coincidental. However, the term 'autistic' had been coined decades earlier to reflect the condition of 'self-absorption' in schizophrenia, and was well-known in the scientific community. Unlike Kanner, Asperger based his observations on hundreds of children, and not surprisingly ended up with a much broader definition of his 'autistic psychopathy'. However, his paper was originally published in German and remained largely unknown in English speaking countries, until four to five decades later, when an account of his work (Wing, 1981) and a translation of his paper (Frith, 1991) were first published in English. His contributions to the field of autism were later recognised by naming Asperger syndrome after him.

Since Kanner's first description of autism, the clinical picture has changed significantly in order to include recent refinements. Recognition of clinical heterogeneity in expression and severity of symptoms led to the redefinition of diagnostic concepts. Wing and Gould (1979) carried out an epidemiological survey in the London borough of Camberwell, investigating children aged 15 years or under, who showed behavioural deficits. From a total of 35,000 children living in this mainly working-class area, 132 were selected as fulfilling one or both criteria for autism. The authors pointed out that social impairments were present in all children, but to a varying degree, and identified what they described as the 'Autism Triad' of impairments. Based on the variability of the impairments, Wing and colleagues proposed the term 'autistic continuum' and later 'autistic spectrum' to reflect the changes in diagnostic criteria, and allow for broader definitions of autism. The early work of Rutter (1978) and Wing (1979, 1981) opened up the field of diagnostic refinements, and significantly influenced changes in categorical definitions of ASDs. The spectrum nowadays includes individuals with symptoms of varying severity, from severely autistic with no speech and low intelligence, to individuals with Asperger syndrome who have normal intelligence and language abilities. However, diagnostic criteria for autism were not included until the third edition of the Diagnostic and Statistical Manual of Mental Disorders by American Psychiatric Association (DSM-III, APA 1980), while Asperger syndrome did not appear in classification systems until the fourth edition (DSM-IV, APA 1994). The heterogeneity of the disorder has been recognised in DSM-V – and Asperger syndrome is no longer separately defined.

Prevalence

Following Kanner's seminal publication in 1943, the scientific community believed that autism was a rather rare condition with a prevalence of around 2-4 per 10,000 (Wing and Potter, 2002). It was not until the 1980s and 1990s that this view was challenged following an annual rise in prevalence rates for the diagnosis of ASD. Studies in the 1990s reported a prevalence of around 10 per 10,000 (0.1%) (Bryson and Smith, 1998), while currently the estimate rose close to 1 per 100 (1%) for all ASDs (Croen et al., 2002; Fombonne, 2009; Lord, et al., 2012; Rice, 2009). The most recent systematic review of epidemiological surveys suggested a median of prevalence estimates of autism spectrum disorders worldwide to be 62 per 10,000 (0.62%) (Elsabbagh, et al., 2012).

The change in reported prevalence led to considerable debate on whether it reflected a real increase or not (Bryson, 1996; Fombonne, 1996). This is because the increase in prevalence coincided with the introduction of autistic "spectrum" in diagnostic manuals. Hence, it was to be expected that the broadening of diagnostic criteria would have an effect on the prevalence of autism. However, there are other factors that could also affect the prevalence rate. These include increased rates of diagnosis due to growing awareness and knowledge among parents and professional workers, development of specialist services, different methods used in epidemiological studies, and lastly the possibility of a true increase in numbers (Wing and Potter, 2002). Wing and Potter (2002) reported that the majority, if not all, of the reported rises in incidence and prevalence rates were due to changes in diagnostic criteria and increasing awareness of ASDs. On the other hand, Bryson and Smith (1998) reflected on revised estimates, and noted that based on the males to females ratio for autism (i.e., 3–4:1), and the number of individuals with typical autism IQ range (50–70 range), which remained unchanged since the early studies, the recent higher prevalence estimates are not due to a fundamental redefinition of autism, but rather to an increased awareness of the heterogeneity of its expression. The notion that environmental factors caused the increase in prevalence (e.g., triple vaccine for measles, mumps and rubella) is today largely rejected, due to recent investigations that failed to support it.

Heredity

Both Kanner and Asperger thought that autism most probably results from a neuropathological origin, based on the observation that symptoms appear very early in life. However, since no organic pathology could be found at the time, this notion was questioned by influential school of American Behaviourism, which explained autistic symptoms in terms of parental ('refrigerator mothers' theory (Bettelheim, 1967)) and early environmental experiences. The first clues to neurobiological underpinnings came from family and twin studies during the 1970s and 1980s, which showed that autism is a highly heritable disorder.

Folstein and Rutter (1977a,b) were the first to undertake a twin study in autism. Despite the small numbers (n=21), their findings showed significant difference in concordance rate between monozygotic (MZ) compared to dizygotic (DZ) twins, implying a strong underlying genetic liability. Furthermore, concordance rates within MZ pairs included a range of cognitive and social deficits and not just the seriously handicapping

condition of autism. During subsequent decades, twin and family studies replicated these findings and yielded further evidence of an exceptionally strong genetic base to the aetiology of autism (Folstein and Rutter, 1977b; Bailey et al, 1995; Ritvo et al, 1985; Rutter, 2000; Steffenburg et al, 1989), with genetic factors explaining over 90% of cases of ASDs according to DSM-IV (Rutter, 2000). Concordance rates for autism are 2 - 6% for siblings and dizygotic twins and approximately 60% for monozygotic twins (Bailey, et al., 1995; Smalley, et al., 1988; Szatmari, et al., 1998). Furthermore, in line with the first study by Folstein and Rutter (1977b), these genetic factors apply to a broader phenotype of autistic spectrum, including milder and not only traditional, more extreme autistic traits (Rutter, 2000).

But are genetic effects overestimated? Does environment have a role in ASDs? A recent study by Croen et al. (2011) showed that prenatal exposure to antidepressant medications (treatment with selective serotonin reuptake inhibitors by the mother) was associated with a 2-fold increased risk of ASD, with the strongest effect was associated with treatment during the first trimester. Although the number of children exposed prenatally to selective serotonin reuptake inhibitors in this study population was low, results suggest that exposure may modestly increase the risk of ASDs. Similarly, other reports point to the importance of environmental prenatal factors for autism risk, such as maternal viral infections during first trimester of pregnancy (Atladottir, et al., 2010), multiple births (Croen et al, 2002), low birth weight (especially for girls) and preterm births (Schendel, et al., 2008), exposure to teratogens such as thalidomide (Miller, et al., 2005), valproic acid (Moore, et al., 2000) and so on. In summary, although ASD is highly genetic, environmental factors may increase the risk of developing ASD.

Triad of impairments

According to the current DSM-IV criteria, individuals have to exhibit six symptoms falling within the three core domains: socialisation, communication, and restricted behaviours, interests, and activities (Witwer and Lecavalier, 2008). Autistic individuals must manifest at least two of the following four symptoms in socialisation: marked impairments in the use of nonverbal behaviours (eye-to-eye gaze, facial expressions, body posture and/or gestures); failure to develop peer relationships appropriate to developmental level; lack of spontaneous seeking to share enjoyment, interests, achievements; and lack of social or emotional reciprocity. Further, they must present with one of the following restrictive/repetitive or stereotypic behaviour, interest, or activity: an encompassing preoccupation with one or more stereotyped and restricted patterns of interest abnormal either in intensity or focus; apparently inflexible adherence to routines or rituals; persistent preoccupation with parts of objects; and motor mannerisms (e.g., hand or finger flapping, twisting, or complex whole-body movements). Finally, they must present with at least one of the following qualitative communication impairments: delay in/total lack of the development of spoken language; in individuals with adequate speech, marked impairments of the conversational abilities; stereotyped and repetitive use of language or idiosyncratic language; and lack of varied, spontaneous make-believe play or social imitative play appropriate to developmental level. In order to obtain a diagnosis of autism, this triad of impairments must be present by the age of three (Witwer and Lecavalier, 2008). It should be noted that any of these symptoms can vary in extent and severity (Bryson and Smith, 1998).

The behavioural manifestation of autism varies according to the severity of the condition, child's developmental level, and intellectual ability. It is known that autism can occur at any point on the IQ continuum, and that IQ is a strong predictor of the outcome of early behavioural interventions (Ben-Itzchak and Zachor, 2007; Volkmar, 2002). The strength and origin of the association between autism and IQ is, however, unclear. Recent studies suggest that this association may largely be explained by genetic factors, however the genetic correlation between extreme autistic traits and intellectual disability was only modest. Extreme autistic traits were substantially genetically independent of intellectual disability (Hoekstra, et al., 2009). But are autistic traits genetically independent among themselves? Marked heterogeneity of behavioural phenotypes in autism leads us to expect substantial genetic heterogeneity underlying autistic symptoms. A large twin study, the Twins Early Development Study found that three core domains disrupted in ASDs (social behaviour, communication and obsessive behaviour) are highly heritable, but independent of each other, at 7 years (Ronald et al., 2005), 8 years (Ronald et al., 2006) and 12 years of age (Robinson, et al., 2011). Results suggested that different genes affect the social and non-social components of ASD (Oliver and Plomin, 2007).

Variation in the clinical picture (high-functioning autism versus Asperger syndrome)

According to the DSM-IV (APA, 1994) and ICD-10 (WHO, 1993) classification systems, HFA and AS are distinguished from each other based on language development. While for HFA diagnosis there have to be delays and/or abnormalities in language functioning, for AS there should be no general delay in language development. Other than this, there is remarkable similarity in behavioural phenotypes between these two subtypes. Both HFA and AS present with the same qualitative impairments in reciprocal social interaction, together with a restricted, stereotyped, repetitive repertoire of interests and activities (Macintosh and Dissanayake, 2004). Whether there are true differences between HFA, which is autism without intellectual disability, and AS is today a topic of debate among clinicians and researchers, with many advocating the use of a dimensional spectrum rather than categorical subtypes (e.g. Lord and Jones,2012; Macintosh and Dissanayake, 2004; Witwer and Lecavalier, 2008). The need to move to dimensional descriptions of autism comes from the recent notion that the relationship between behavioural phenotypes and clinical diagnosis is not a clear-cut one. Clinical distinctions across subtypes can very even across sites with well documented fidelity using standardized diagnostic instruments (Lord, et al., 2012). Witwer and Lecavalier (2008) reviewed 22 studies published between 1994 and 2006 that examined the differences between the subtypes of autism spectrum disorders, and suggested that the results largely did not support the differences between HFA and AS. Rather, they suggested that the most salient group difference was not overall language delay, but the category of IQ, drawing the question on how linguistically different HFA and AS groups are (for more on linguistic differences between these two groups see Section 5.3). Other reviews of epidemiological studies and empirical research have also not provided clear evidence for the validity of the HFA and AS subtypes (Macintosh and Dissanayake, 2004). Nevertheless the distinctions between the two subtypes (if they exist) may be important, since they might have implications for determining the aetiology of the condition.

5.2 Language deficits in autism: behavioural studies

Although language and communication deficits in autism are its defining feature, they can vary from being striking to hardly noticeable. This section will give a brief overview of linguistic processing in autistic individuals.

Parents of autistic children know how daunting communication impairments can be, since communication is essential in our social world. It is therefore appropriate to start with the opening lines of The Siege, written by Clara Claiborne Park (1972, quoted in Crystal and Varley, 2006, p.14) who is a parent of an autistic child. In this she gives a short description of the communication impairments she observed in her 18 months old child.

"One speaks to her, loudly or softly. There is no response. She is deaf, perhaps. That would explain a lot of things - her total inattention to simple commands and requests, which we thought stubbornness; the fact that as month follows month she speaks no more than a word or two, and these only once or twice a week; even, perhaps, her self-absorption. But we do not really think she is deaf. She turns, when you least expect it, at a sudden noise. The soft whirr as the water enters the washing machine, makes her wheel round. And there are the words. If she were deaf there would be no words. But out of nowhere they appear. And into nowhere they disappear; each new word displaces its predecessor. At any given time she has a word, not a vocabulary."

Autism, as a neurodevelopmental disorder, has profound consequences on language and general communicative ability. Previously, studies suggested that up to fifty percent of children with autism were nonverbal (Lord and Rutter, 1994). However, recent research indicated that this percentage is much lower. Lord, Risi, and Pickles (2004) found that at 9 years old only 14–20% of autistic children were nonverbal (defined as a daily use of five or fewer words). Recent research agrees that approximately one-third of individuals with autism never develop functional use of language (Fletcher, et al., 2010), with communicative deficits extending to non-verbal domains, such as impoverished use of eye contact and gestures. As described in the extract above, the response to language can be so minimal during early childhood that some children with autism seem deaf although their hearing is normal. In the remaining two thirds language does develop, but in many cases it is represented by deviant and unusual language and speech forms. Some argue that these children, who use verbal communication, are better described in terms of language delay than deviance, a delay that Tager–Flusberg (2004) suggests is similar to the one seen in specific language impairment (SLI). However, delay in spoken language is observed only in half of ASD individuals (Alarcon, et al., 2002; Spence, et al., 2006). Therefore it needs to be remembered that there are significant differences in the extent and quality of linguistic symptoms observed among the ASD population. Furthermore, as Galaburda et al. (2002) notes, different forms of language have different developmental schedules, and thus break down separately in developmental disorders. Therefore, different aspects of verbal and non-verbal language may be differently affected in autistic individuals.

Language and global developmental deficits

Communicative impairments of autistic individuals have to be considered in the context of global developmental delays and deficits that co-occur. Language is tightly linked to other cognitive processes (learning, memory, attention, perception, reasoning, etc.), and failing to examine the broader cognitive picture might result in omission of crucial information regarding communicative problems in autism. For example, if selective attention is impaired, it might lead to impairment in receptive language skills. Today we are aware that impairments linked to communication and language deficits in autism include pre-linguistic pathology (cognition), pragmatic disability (saying the wrong thing at the wrong time), motor programming (apraxia), motor execution (disorder of articulation/voice - monotone, flat, mechanic), reception (due to attention) and message decoding problems (Crystal and Varley, 2006). So how does early language link to each of the core impairments characteristic of autism spectrum disorders?

Dworzynski, et al. (2007) used a population-based twin sample and prospectively assessed at 2, 3, 4 and 8 years to determine the extent to which shared genetic and environmental factors underlie the association between early language abilities and autistic-like traits (ALTs) in middle childhood. ALTs measured by the Childhood Asperger Syndrome Test at 8 years were explored in relation to language assessed earlier by the MacArthur Communicative Development Inventory at 2, 3 and 4 years. Their results supported the idea that the triad of core features in ALTs are aetiologically heterogeneous, and furthermore that early language is genetically related to social and communication impairments but not to restrictive and repetitive behaviours and interests. It seems that shared genetic influences drive language performance to be an early antecedent of later ALTs. This is in line with a population-based study by McEwin, et al. (2007) showing significant correlation between imitation, vocabulary, pretend play, and socially insightful behaviour in healthy 2-year-old twin pairs. Therefore, the development of language seems to be linked to development of various social skills, and therefore has implications when discussing the impairments present in autism.

Studies investigating the comparison between language and nonverbal cognitive skills gave further support to the notion of congruence between the two (Lord, et al., 2004). However this congruence is not present in early years, when a discrepancy between verbal and non-verbal cognitive skills is present (Rice, et al., 2005). This is supported by the findings of Joseph, et al. (2002), who reported that more preschool children with autism had verbal scores below nonverbal scores, compared to school-age children. Furthermore, whether a child is verbal or nonverbal affects this relationship. For nonverbal children with autism, only 16% showed a discrepancy between verbal and nonverbal cognitive skills (Lord, et al., 2004). These findings suggested that for a majority of nonverbal autistic children linguistic delays were expected based on their nonverbal cognitive performance. Also, Tager–Flusberg (2004) found that most verbal children with autism had normal nonverbal intelligence. Therefore, at least for young children with autism and older, verbal children with autism, there is a congruence between language and nonverbal cognitive skills (Rice, et al., 2005).

Development of lexical knowledge and morphosyntax

Lexical development has been extensively studied in children with autism. One of the surprising findings involves a subgroup of children with autism (around 20 percent) who show normal lexical development followed by an abrupt decline in the use of words (Lord, et al., 2004). Language loss group differs significantly from the majority of autistic children that display a delayed onset of the first words, highlighting the clinical heterogeneity of the autistic population. Investigations of the morphosyntactic abilities in verbal children with autism report that approximately 67% of verbal children show mixed expressive and/or receptive language delay (Allen and Rapin, 1980, 1992). Morphosyntactic deficits observed in spontaneous speech include omission of the finiteness morphemes, errors in eliciting past tense and third person singular verbs etc. (Roberts, et al., 2004).

Pragmatics in autism

Pragmatic impairments, which encompass social aspects of communication, are a defining hallmark of autism. Despite the clinical heterogeneity of the autistic population, and the presence or absence of linguistic pathology, all individuals with autism show some pragmatic impairment (Rice, et al., 2005). These deficits vary from restricted range of speech acts (Loveland, et al., 1988; Wetherby, 1986), conversational deficits (Loveland and Tunali, 1993; Tager–Flusberg and Anderson, 1991; Tager–Flusberg and Sullivan, 1995) to deficits in understanding mental states of others in a conversation (Loukusa, et al., 2007; Paul and Cohen, 1984; Perkins, et al., 2006). Autistic individuals often fail to respond directly to conversational stimuli, and usually end up keeping a monologue of their own. Hence, an 'apparent' conversation is obtained. The following extract is taken from Crystal and Varley (2006, p.161) to illustrate this point.
The conversation is taking place between an autistic child and his therapist.
T- what are you going to do with that car now?
C- I like my car (pushing it on the floor).
T- look, I've got one like that!
C- in here it goes (pushing car into garage).
T- don't forget to shut the doors.
C- find the man now (looking about)...

Semantics in autism

Subtle abnormalities in semantic processing are often found among different subtypes of autistic spectrum, including difficulties in non-literal language and language context (Verhoeven, et al., 2010). Semantic impairments result in autistic subjects showing no difference when recalling semantically encoded compared to perceptually encoded words, and reproducing to an equal extent concrete words compared to abstract ones (Verhoeven, et al., 2010). Behavioural findings were strengthened by a recent functioning imaging study, which showed diminished differential fMRI activation patterns when contrasting concrete and abstract word stimuli in autism (Harris, et al., 2006).

5.3 Categorical versus dimensional approach to language and communication deficits in autism

Although the new edition of DSM (DSM-V) will drop the categorical distinctions and combine all the subtypes into one autism spectrum disorder, in my data analysis I have used both categorical and dimensional approaches. Hence, this section will briefly introduce the categorical and dimensional approach to language and communication deficits observed in autism.

The categorical approach states that language delays and/or language functional abnormalities have to be present for a diagnosis of HFA, while in comparison there is no significant delay in language acquisition in Asperger's syndrome. Studies that examined language and communication skills found that AS individuals scored significantly higher on language measures such as expressive language skills (Ghaziuddin and Gerstein, 1996; Ozonoff, et al., 2000; Szatmari, et al., 1995), pedantic speech (Eisenmajer, et al., 1996; Ghaziuddin and Gerstein, 1996), phonemic fluency (Spek, et al., 2009) but not on measures of receptive language skills (Ozonoff, et al., 2000; Ramberg, et al., 1996), semantic fluency (Spek, et al., 2009), conversational impairments (Fine, et al., 1994) and pragmatic language (Verte, et al., 2006b) where no difference was found between the subtypes. Inconsistent results were reported for the measures of echolalia (Eisenmajer, et al., 1996; Fine, et al., 1994; Miller and Ozonoff, 2000; Szatmari, et al., 1989), repetitive speech (Eisenmajer, et al., 1996; Szatmari, et al., 1989), and flat/mechanical intonation (Eisenmajer, et al., 1996; Fine, et al., 1991; Gillberg, 1989). When exploring verbal IQ, it seems that the distinction between AS and HFA rests largely upon diagnostic and inclusion criteria. Studies that followed the DSM-IV diagnostic criteria found significantly higher verbal IQ in those with AS compared to HFA (Klin et al. 1995; Miller and Ozonoff 2000; Ghaziuddin and Mountain-Kimchi 2004), while studies that modified DSM criteria and widened age and IQ range observed no differences (de Bruin et al. 2006; Ozonoff et al. 2000). Overall, the findings indicate that distinction between those with HFA compared to those with AS on language measures are inconsistent, and seem to be largely dependent on the IQ factor, age and/or related to the original diagnostic criteria (Macintosh and Dissanayake, 2004; Witwer and Lecavalier, 2008). Age is especially important in altering linguistic distinctions between HFA and AS individuals, since differences in communication impairments between these two groups seem to diminish over time (Eisenmajer, et al., 1996; Gilchrist, et al., 2001; Howlin, 2003; Joseph, et al., 2002; Verte, et al., 2006a). If the categorical approach is valid, then the question is how individuals with HFA overcome these language differences - whether this is due to compensation mechanisms or simple developmental factors is not known (Macintosh and Dissanayake, 2004). However, future changes in diagnostic criteria (DSM-V) suggest that linguistic differences between these two subtypes are not meaningful and well-defined (Macintosh and Dissanayake, 2004; Witwer and Lecavalier, 2008). This dimensional approach gets further support from studies reporting that children with AS can also have difficulties in language development (Eisenmajer, et al., 1996; Prior, et al., 1998), and that not all children with HFA experience language delay ((Eisenmajer, et al., 1996; Miller and Ozonoff, 2000). This PhD study will use both, the categorical – to acknowledge the historical split between HFA and AS; and dimensional – to explore the anatomical differences across the whole autistic sample.

5.4 Conclusion

This Chapter offered an introduction to autism spectrum disorders and underlying language and communication deficits, discussing both categorical and dimensional approach to this disorder. What is the biological basis for the observed language deficits? The following Chapter 6 will discuss the neuroanatomical findings in autism and how they might link to language deficits, and importantly introduce the third diffusion tractography study of this PhD project, investigating perisylvian language pathways in ASD.

Chapter 6

Perisylvian language pathways in Autism Spectrum Disorder

6.1 Introduction and General Aims

Autism spectrum disorders (ASDs) have become the focus of intense research in the last couple of decades, and yet remarkably little is known about the underlying neural mechanisms that cause autistic behaviour. The aetiology of autism remains elusive, and presently there is no biological marker for autism. This Chapter attempts to give an overview of the recent neuroanatomical findings in ASD, focusing on the brain language regions. It has been suggested that abnormal brain connectivity and white matter development may underlie some of the communication deficits observed in ASD (Belmonte, et al., 2004; Herbert, et al., 2004). However, direct evidence linking the severity of communication deficits with anatomical abnormalities is missing.

The present study has applied diffusion tractography to dissect 'social' and language pathways (specifically the three segments of the arcuate fasciculus) in adults with ASD and matched controls with normal intelligence. The primary hypothesis was that people with ASD have structural abnormalities in the left perisylvian pathways specialised for language, social cognition and theory of mind compared to controls. I also predicted greater white matter abnormalities in those patients with the most severe clinical deficits in communication. This analysis of anatomy and behaviour was explored in different ways: categorically (high-functioning autism versus Asperger syndrome), dimensionally (within ASD) and by investigating across the whole sample the relationship of brain to language ability.

6.1.1 Unravelling the brain in autism

Both Kanner and Asperger thought that the origin of autism lies within the field of neuropathology, on account of early appearance of symptoms. However, the idea of an organic cause was largely forgotten during the following decades, in the light of a wide-spread bias in American psychiatry that all psychiatric disorders result from inadequate parenting and distressing early experiences. This belief was further supported by no direct evidence of brain abnormality and a lack of proper methodology to study the brain at the time. After six decades of research, the evidence of a neuropathological contribution is indisputable, yet definite biological markers have not been identified. This section will try to give an overview of the recent research and build up a coherent picture of what really happens in the autistic brain. The emphasis will be placed on white matter abnormalities and diffusion tensor imaging research.

6.1.1.1 Molecular and structural evidence

Developmental abnormalities were first indirectly noted in Kanner's original paper from 1943. He described 11 children with autism, and noted that five of them had 'large heads'. Today referred as macrocephaly, it represents one of the most prominent theories of neuropathology in autism. Structural MRI studies consistently found an increase in overall brain volume (Aylward, et al., 2002; Courchesne, et al., 2001; Hazlett, et al., 2005; Lainhart, 2006; for a review see Amaral, et al., 2008). In a large epidemiological study, 14% of autistic subjects had macrocephaly, 11% of males and 24% of females (Lainhart, et al., 1997). The study did not find any association between abnormal head growth and behavioural phenotypes, such as nonverbal IQ, verbal status, neurological soft sings or minor physical anomalies in the autistic subjects. Taking all the recent findings together, abnormally accelerated brain growth was observed in 50-70% of children with autism during the first 2 years of life (Courchesne, 2004; Courchesne and Pierce, 2005; Lainhart, 2006; Redcay and Courchesne, 2005). According to the 'brain growth dysregulation hypothesis', this excessive age-related growth in postnatal life is followed by an apparent arrest, resulting in no significant difference between autistic and healthy population in adulthood.

The question that intrigued the scientific community was whether excessive brain growth equally affects cortical grey and intra-cerebral white matter. Herbert, et al. (2003) suggested that there is a disproportionate overgrowth in the white matter, compared to the grey matter. Also, as Conturo (2008) argued, the increase in cortical grey matter might be linked to the increase in white matter by an increase in white matter projections necessary to maintain the connectivity of the increased number of cortical cells reported in histopathological studies (Casanova, 2002, 2003, 2006). However, in a recent review article Amaral et al. (2008) summarised imaging findings and concluded that there were no significant differences between grey and white matter volumes in autism, with both exhibiting significant increases compared to healthy controls, that dissipate over time. What is more, it seemed that the difference in volume was declining faster in white matter as compared to grey matter volume over time. Nevertheless, regional differences do seem to be present, with the majority of studies reporting the frontal lobes to be predominantly affected (Hazlett, et al., 2006; Carper, et al., 2002; Herbert, et al., 2003, 2004).

Neuropathological findings in autism

In order to really understand the neurobiology of the observed neuroanatomical differences, we need to turn to the neuropathological data. If the brain volume is bigger in autism, does it mean there are more neurons, axons, glia, or synapses? Which neuropathological processes are behind excessive brain growth? Unfortunately, there are insufficient data from neuropathological studies to clarify which direct mechanisms might underlie this phenomenon. However, studies do point to early pre- and postnatal developmental abnormalities affecting multiple brain regions, such as frontal cortices, the limbic system, and cerebellum.

To date, subtle neuronal abnormalities have been found throughout the cortex in autism, affecting predominantly the frontal lobes and limbic system. Studies showed reduced size and spacing of cortical radial minicolumns (Casanova, et al., 2002); increased packing density of cells and reduced cell size in the anterior cingulate gyrus, hippocampus, entorhinal cortex, mammillary body, subiculum and amygdala (Kemper and Bauman, 1985, 1994, 1996); fewer neurons in total amygdala (Schumann and Amaral, 2006); increase in the number of Von Economo neurons in the anterior cingulate cortex and frontoinsular cortex (Santos, et al., 2010); and minor disruption of dendritic orientation with reduced branching of pyramidal neurons in autism (Raymond et al,1995). Furthermore, Bailey, et al. (1998) observed thickened cortices, irregular laminar patterns, poor grey-white matter boundaries, areas of increased neuronal density and abnormally oriented pyramidal cells. Cerebellar abnormalities were found as well, reflected in reduced density of Purkinje cells (Bauman, 1991; Vargas, et al., 2005; Williams, et al., 1980; Ritvo, et al 1986) and reduced sizes of Purkinje cells (Fatemi, et al., 2002). However, abnormalities reported in these neuropathological studies were not always replicated and mostly occur without correlations with the severity of autistic symptoms.

Overall, post-mortem evidence, based on small sample numbers, points to distributed atypical development of the autistic brain. This is in line with the findings by Fatemi, et al. (2001a, b) reporting 40% reduction in Reelin, a signalling protein involved in neuronal migration and lamination, and 34-51% reduction in Bcl-2, a protein responsible for apoptosis, and thus cell density. Verhoeven, et al. (2010) suggested that abnormal brain patterning seen in autism resembles the earlier stages of brain maturation, thus reflecting the features of an immature brain.

Nevertheless, we have to be aware that interpretations from neuropathological findings face many limitations. First, a dearth of human pathological material affected the quality of results, with the small samples mostly involving older children and adults, thus being inadequate to investigate directly abnormal development in autism. Secondly, there are technical limitations intrinsic to post-mortem studies. For example, Whitney et al. (2008) found no significant difference in the density of Purkinje cells between the autistics and controls when using more accurate method of cell counting (immunostained for calbidin-D28k), suggesting that instead of observing cell reductions, previous studies might have simply lacked the technical tools to accurately count the cells. Thirdly, the paucity of animal models is preventing direct exploration of biological processes involved in autism. And lastly, post-mortem studies largely included brains of autistic patients with other co-morbid disorders. Nowadays, it is increasingly recognised that ASDs are associated

with other conditions, such as epilepsy, attention-deficit hyperactivity disorder, obsessive compulsive disorder, mood disorders and so on. Epidemiological studies indicate that 25–30% of individuals with autism have associated medical conditions, which predominate among the most severely intellectually disabled (Bryson and Smith, 1998). This represents the biggest limitation of recent post-mortem findings, since they included clinically heterogeneous samples with other associated medical conditions that also affect brain development. For example, from 24 post-mortem studies of cerebellum, 19 (or 79%) show decreased density of Purkinje cells. However, 22 of the 24 brains examined in post-mortem studies came from individuals with mental retardation, while almost half had epilepsy (for a review see Amaral, et al., 2008). Hence, it is possible that the abnormalities of Purkinje cells is a secondary phenomenon linked to co-morbid disorders, rather than primary phenomenon of autism. The post-mortem findings must be considered with caution, as we cannot decipher the association if more than one disorder is present.

General disruption of brain development: clues from genetics

Today it is largely accepted that inherited brain anomalies are pivotal in autism aetiology. However, deciphering the genetics of autism remains challenging, since it involves multiple, rare genetic variants and complex gene-environment interactions. Environmental factors, such as prenatal exposures to teratogens (Arndt, et al., 2005), also play a role by interacting with genetic susceptibility to increase the risk of ASDs. At present, at least 12 genome scans are completed, many chromosomal regions have been implicated (e.g., 2q, 3q, 7q, 16p or 13q21) and over 100 candidate genes studied - however few results have been replicated (Barnby & Monaco, 2003; Geschwind and Levitt, 2007) since a ‘single cause’ approach is too simple to account for the clinical heterogeneity in autism (Bishop, 2006).

Genetics points to an overlap between autism and a general disruption of brain development, and implicates processes such as neuronal migration, cellular proliferation, synaptic connectivity and so on, as being abnormal in autism. Research revealed mutations of genes responsible for:

- synaptogenesis, e.g. Neuroligin 3 and Neuroligin 4 genes (Jamain, et al., 2003);
- neuronal migration, e.g. contactin-associated protein-like 2 (CNTNAP2) gene (Strauss, et al., 2006);
- dendritic development, e.g. Shank3 gene (Durand, et al., 2007);
- cerebellar developmental patterning, e.g. Engrailed 2 gene (Yang, et al., 2008);
- cellular growth and proliferation, e.g. PTEN gene (Varga, et al., 2009);
- development of local and long-range cortical circuits, and the cerebellum, gene encoding the tyrosine kinase receptor MET (Campbell, et al., 2006);
- synaptic and neuronal signalling function, downregulation of multiple genes (Voineagu, et al., 2011).

A recent study by Voineagu et al. (2011) further supported the notion of strong molecular abnormalities in ASDs, implicating transcriptional and splicing dysregulation as underlying mechanisms. Regional patterns of gene expression that typically distinguish frontal (BA09) and temporal cortex (BA41, BA42) during foetal development were significantly attenuated in ASD, pointing to abnormal developmental patterning as a potential pathophysiological driver. The number of genes showing significant expression differences between

frontal and temporal cortex was 510 in controls and only 8 in autism. Nevertheless, many features reflecting the general organisation of the autistic brain transcriptome were otherwise consistent with that of the normal brain (87%) (Voineagu et al., 2011).

Taking all the genetic findings together, there is strong evidence that risk-associated candidate genes for autism are linked to disruption in the development of brain connectivity. Brain connectivity is used as a general term, referring to a number of factors, such as synaptic and neuronal signalling function, signal transduction, physical properties of neurons, axons, synapses, and lastly development of local and long-range circuits and brain pathways. Aberrant brain connectivity in autism received support from many studies using various methods, like functional magnetic resonance imaging, diffusion tensor imaging, electroencephalography and so on, ultimately leading to a 'developmental disconnection' model of autism, implying over-connectivity of local connections (particularly within the frontal lobes) and under-connectivity of long-range connections along the anterior-posterior axis (Courchesne, 2005; Geschwind and Levitt, 2007).

6.1.1.2 Developmental disconnection syndrome

A unifying model that explains brain abnormalities found in autism was proposed by Geschwind and Levitt (2007), in which higher-order association areas of the brain that normally connect to the frontal lobe are partially disconnected during development (referring to both under-connectivity and over-connectivity) . This concept of 'developmental disconnection syndrome' clarifies behavioural phenotypes seen in autism in the context of a heterogeneous aetiology.

The model describes how early disruption of maturational schedules, evidenced by early brain overgrowth and neuropathological findings of developmental abnormalities, results in an aberrant connectivity and dysfunction that subsequently leads to the development of autistic behavioural impairments. These disconnections disrupt the development of important neural circuits, hindering the formation of social and communication skills, and leading to repetitive and stereotyped patterns of behaviour in autism. For example, involvement of the dorsolateral prefrontal cortex and anterior cingulate cortex is predicted to disrupt joint attention, which is necessary for later development of language and social cognition (Geschwind and Levitt, 2007). Based on which systems are disconnected, and how severe and widespread this disconnection is, it has been proposed that this leads to distinct autistic phenotypes.

Emerging 'disconnection' explanations, suggesting wide-spread disturbances of brain connectivity, were supported by functional MRI studies. For example there are numerous reports of a significant reduction in functional correlation among various cortical regions activated during various executive, social, and communication tasks in autism as compared to controls (Just, et al., 2004, 2007; Kana, et al., 2006, 2007; Koshino, et al., 2005; Luna, et al., 2002; Mason, et al., 2008; Schultz, et al., 2000). Overall, findings point to reduced functional connection or disconnection between different cortical areas essential for higher order processing functions in autism.

Some authors have related neuropathological findings with resulting aberrant connectivity in autism. Courchesne and Pierce (2005) suggested that autism is the underdevelopment of large integrative and projecting pyramidal neurons, especially those in the frontal cortex. They argued that long distance under-connectivity but local over-connectivity would appear as a result. However, relating neuropahological findings based on small heterogeneous samples involving low-functioning autism with associated medical conditions, and imaging findings based mostly on high-functioning autism without co-morbid disorders is problematic.

In the light of recent 'disconnection' theories the study of white matter is becoming increasingly more important, especially after the findings of abnormal integrative processing involving intrahemispheric as well as interhemispheric transfer of information (Just, 2004; for the recent review see Amaral, 2008). Hence white matter abnormalities are discussed in more detail in the following subsection.

6.1.1.3 White matter abnormalities in autism

Diverse neuroimaging and neuropathological findings have indirectly suggested that aberrant white matter connectivity is an important contributor to the core triad of autistic impairments (for example see Belmonte, et al., 2004; Just, 2004; Minshew, 1996; Minshew and Williams, 2007)..

However, the results from in vivo imaging studies of white matter have been variable.. Some published studies revealed more severe white matter alterations in the right hemisphere (Barnea-Goraly, 2004 etc), whilst others found severe white matter deficits in the left (Ben Bashat, et al., 2007; Pardini, et al., 2009 etc) of autistic subjects. McAlonan et al. (2009) argued that the asymmetry and localisation of anatomical abnormalities in autism is dependent on the specific behavioural impairment studied. For instance, she reported that HFA is characterised by volumetric deficits localised mainly in the left hemisphere, while AS is associated with abnormalities in white matter systems predominantly in the right hemisphere. Some suggested that anatomical abnormalities in autism affected predominantly the outer white matter radiations containing association tracts (Herbert, et al., 2004), whereas others argued that the most affected were internal regions containing mainly projection fibres (McAlonan, et al., 2009; Rocha Brito, 2009). More recent imaging evidence points to a diffuse pattern of white matter abnormalities present at the level of both association and projection white matter pathways of adults with autism spectrum disorder (Ecker, et al., 2012). However, these voxel based morphometry studies could not localise the abnormalities to specific tracts and further lacked the power to examine the microstructural integrity of white matter connections. With the advent of diffusion tensor imaging (DTI) it became possible to study the specific white matter pathways in the autistic brain. The following section will give an overview of the recent DTI findings in ASD.

Diffusion tensor imaging in autism

DTI provided the opportunity to explore white matter in autism, and DTI tractography to trace specific fibre pathways and test for altered physical connectivity between brain regions. So far, there has been a substantial number of diffusion studies in autism, compared to only nine using tractography to dissect language white matter tracts.

DTI studies highlighted that individuals with ASD have abnormalities in important white matter regions involved in social cognition (fusiform gyrus, superior temporal sulcus) and theory of mind tasks (ventromedial prefrontal cortex, anterior cingulate, temporo-parietal junction, superior temporal sulcus and amygdala), long-range communication processing (anterior corona radiata, right retrolenticular part of internal capsule) and interhemispheric transfer of information (corpus callosum) (Rocha Brito, et al., 2009; Alexander, et al., 2007; Barnea-Goraly, et al., 2004; Keller, et al., 2007; Thakkar, et al., 2008; Lee, et al., 2007; Ben Bashat, et al., 2007).

Tractography studies focused on alterations of specific white matter pathways underlying the three core domains of autistic dysfunction. Abnormalities were found in the regions of social cognition (limbic tracts, frontal, temporal, occipital and cerebellar connections), language (fronto-temporal association pathways) and repetitive and stereotyped behaviour (frontal connections and fronto-striatal network) in both hemispheres. Hence, I will now focus on regions and pathways underlying language and communication.

6.2 Language-related brain research in autism

Recent imaging studies have made big advances in delineating the neuropathology of ASDs, however, relatively few have focused on understanding the neural basis of language and communication deficits. The reason possibly lies in the challenging nature of language-related research, due to language impairments being one of the most variable and complex symptoms of ASDs - occurring along many levels: phonological, syntactic, semantic and pragmatic. So far, research has suggested that language impairments in ASD are attributable to the maldevelopment of multiple brain regions and multiple underlying structural and functional networks, all contributing to the final linguistic phenotype. Findings in this area are of great importance, since the investigation of specific autism-related impairment, such as language deficits, could be more informative than investigations based on the categorical ASD subtypes. This section will give an overview of up-to-date research of language-related brain regions in autism, focusing particularly on the abnormalities of perisylvian language pathways, providing a context for understanding the importance of this PhD project.

6.2.1 Language-related cortical regions

Research points to both structural and functional alterations of language-related cortices in ASD. Not surprisingly, language-related cortical abnormalities of the frontal and temporal lobes have been consistently found. Neuropathological studies pointed to diffuse cortical abnormalities, such as alterations of the cortical minicolumns (Casanova et al., 2002, 2003) and cortical dysgenesis of the frontal and temporal lobes (Bailey et al., 1998) as a possible source of aberrant connectivity underlying language dysfunction in ASD. Further support for frontal and temporal lobe abnormalities in autism came from neuroimaging studies. A small but consistent increase in grey matter volume of the left middle and inferior frontal gyri (BA46 and BA10) important for language processing was found after quantitative meta-analysis of voxel-based morphometry studies (Via, et al., 2011). The inferior frontal gyrus is a structure previously implicated in language dysfunction (Groen et al, 2008) and mirror neuron system dysfunction in autism (Dapretto et al, 2006; Oberman et al, 2005; Uddin et al, 2008). These findings converge with previously reported larger frontal language region volumes in both children and adolescents with ASD (Knaus, et al., 2009). Furthermore, in children with ASD, a larger left pars triangularis and left frontal language volume were associated with more severe communication and social autism symptoms. This finding may be related to suggestions that a lack of pruning, especially in the right hemisphere, contributes to language dysfunction in autism (Beaton, 1997).

Besides frontal, temporal language areas were also found to be affected in autism, with some studies reporting an increase and others a decrease in temporal grey matter volume. A decrease in grey matter density was observed in the left planum temporale (Rojas, et al., 2002), left superior temporal sulcus, left inferior temporal and supramarginal gyrus (Hadjikhani et al. 2006; Hardan et al. 2006; Wallace et al. 2010), and bilateral superior temporal gyri (Boddaert, et al., 2004), while grey-matter increases were reported for primary and associative auditory cortex (Hyde et al. 2010). Moreover, a disruption of structure–function relationship between superior temporal gyrus' volume loss and receptive language function was observed in ASD (Bigler et al., 2007). The relationship between classical cortical language areas in autism also seems to be impaired, with autistic children showing reduced correlations between gray matter volumes in frontal and temporal language regions compared to controls (McAlonan et al., 2005). Furthermore, alterations in asymmetry patterns were observed for brain volumes of both the frontal and temporal lobes in ASD (Rojas et al., 2002, 2005; Herbert et al., 2005). Studies also reported hemispheric asymmetry alterations in Broca's area (De Fosse et al., 2004; Herbert et al., 2002; Tager-Flusberg and Joseph, 2003), planum temporale and Heschl's gyrus (Rojas et al., 2002, 2005). Children with autism present with less lateralised cortical structures compared to neurotypical subjects, and the loss of left asymmetry for the frontal and temporal language areas. It is reported that this loss of leftward asymmetry is due to an increase in the rightward asymmetry, which may result from an early abnormal brain growth during development (Herbert, et al., 2005).

There is also some evidence that, within ASD, anatomy of language regions may differ between those with HFA and AS. A recent meta-analysis of MRI studies reported that distribution and direction of anatomical differences was different between HFA and AS (Yu, et al., 2011) . AS involved clusters of lower grey matter volume in the right hemisphere and clusters of greater grey matter volume in the left hemisphere. On the other hand, autism led to more extensive bilateral excess of grey matter. Both conditions shared clusters of grey matter excess in the left ventral temporal lobe. The authors suggest that the difference in language acquisition drives anatomical differences between HFA and AS. However, another meta-analysis found no significant differences in regional gray matter volume between the two groups (Via, et al., 2011). It is still not clear whether subtle differences in language dysfunction can alter the brain's anatomical basis. How language dysfunction affects functional connectivity in the brain will be discussed next.

6.2.2 Altered functional connectivity underlying language processing in autism

Two key areas that become active during language processing are left inferior frontal gyrus, or Broca's area (BA's 45–47) responsible for sentence comprehension through syntactic and semantic processing and working memory functions, and left superior and middle temporal gyrus, or Wernicke's area (BA21) involved in lexical processing (Bookheimer, 2002). Functional activations of these cortical areas, together with their interhemispheric synchronization and hemispheric lateralisation, are often altered during language processing tasks in ASD. However the picture is sometimes mixed, and inconsistencies remain regarding the nature of language processing anomalies in the condition.

One of the first functional neuroimaging studies on language processing in autism was carried out by Just, et al. (2004) who investigated sentence comprehension. HFA subjects performed worse on sentence comprehension tasks, and exhibited reduced activation of Broca's area with increased activation in Wernicke's area compared to controls, revealing weakness in the integration of the meaning of words into a coherent syntactic structure. This finding gave further evidence that processing of low-level linguistic tasks (single words) is well preserved in children with autism, while the processing of higher-level tasks requiring integration (meaning of complex sentences) is often impaired (Goldstein, et al., 1994). Consistent with this hypothesis, individuals with autism performed more poorly and demonstrated increasingly more abnormal evoked-potential patterns on listening tasks as spectral and dynamic complexity increased (Samson et al. 2006). This can be explained in terms of the recent findings reporting anomalies of neural mechanisms indexing sound discrimination in young autistic children (Kuhl et al. 2005) and poor attention to phonemes (Ceponiene et al. 2003). Although it was proposed that autism is a disorder caused by under-functioning of higher-level integrative circuitry (Just, et al., 2004) recent findings question this. Scott-Van Zeeland, et al. (2010b) showed that even basic aspects of language acquisition are typically impaired in autism. The authors studied statistical learning, which refers to identification of word boundaries in continuous speech, and observed decreased sensitivity to the statistical and speech cues available in HFA subjects. However, it is not clear whether the results reflect true deficits in language acquisition, or are a product of the contrived nature of this experimental design. Therefore, further studies are needed to demonstrate whether basic aspects, and not just higher-level processing of linguistic information are impaired in autism.

A lower degree of information integration entails lower synchronization across different cortical networks responsible for language processing. Just, et al. (2004) demonstrated lower functional connectivity, i.e. the degree of synchronization or correlation of the time series of the activation, between the various participating cortical areas important in language processing in the autistic adults. Likewise, toddlers with autism showed significantly weaker inter-hemispheric synchronization in the putative language areas (Dinstein, et al., 2011). The strength of this synchronization was positively correlated with verbal ability and negatively correlated with autism severity. Decreased functional connectivity was also found for working memory in high functioning autism (Koshino, et al., 2008). Taken together, results converge with the 'under-connectivity' theory on tasks involving the semantic and associative networks (Beversdorf et al., 2000).

It is important to note that processing mechanisms for speech versus song are differently affected in autism. While activation in inferior frontal gyrus is reduced in autistic children relative to controls during speech stimulation, it was greater than controls during song stimulation (Lai, et al., 2012). Furthermore, functional connectivity for song relative to speech was also increased between left inferior frontal gyrus and superior temporal gyrus in autism, and large-scale connectivity showed increased frontal-posterior connections (Lai, et al., 2012). Preference for song stimuli in autism might reflect greater reliance of the right hemisphere, noticed in several imaging studies that revealed atypical cerebral language lateralisation in autism. HFA individuals showed more extensive activation in homologous areas of the right hemisphere during sentence comprehension tasks (Kleinhans et al., 2008) and smaller degree of lateralisation during semantic processing of words (Knaus, et al., 2008). Hence, it is possible that the right hemisphere will not be affected in this PhD study and that anatomical alterations will be found only in the left hemisphere.

Studies further showed that due to decreased activation within inferior frontal gyrus there is a compensatory higher activation in the left temporal regions (Harris et al., 2006; Just, et al 2004). Nevertheless, temporal regions in autism were found to be both over- and under-connected (Castelli, et al., 2002). Also, the structure consistently implicated as abnormal in autism is the superior temporal sulcus (STS), involved in language, biological motion and theory of mind tasks (Redcay, et al., 2008; Groen, et al., 2008; Klin, et al., 2009). Abnormalities of the STS were found in several anatomical studies (Carper, et al., 2002; Hardan, et al., 2006; Hazlett, et al., 2006; Casanova, et al., 2002). Decreased speech processing ability of STS region was noted in the lack of activation in response to vocal sounds (Gervais, et al., 2004), reduced activation for socio-communicative tasks in positron emission tomography (Castelli, et al., 2002) and functional MRI studies (Kana, et al., 2006; Shih, et al., 2011), with reduced synchronization with regions important for social processing (Kana, et al., 2009; Kleinhans, et al., 2008*;* Mason, et al., 2008). These functional alterations in autism were recently explained in terms of atypical maturation of STS. Shih, et al. (2011) suggested that altered trajectories for functional segregation and integration of networks in autism are result of aberrant anatomical maturation of STS.

Overall, the studies report aberrant functional 'connectivity' between key language regions. Whether this altered functional connectivity indicates differences in anatomical organisation of language in autistic brain will be discussed in the following section.

6.2.3 Altered structural connectivity underlying language in autism

White matter abnormalities affecting language-related areas have been frequently observed in ASD, however results are somewhat inconsistent. Some studies revealed white matter volume increases in tracts known to be important for language, such as arcuate fasciculus, inferior fronto-occipital and uncinate fasciculi (for a meta-analysis of voxel-based morphometry studies see Radua, et al., 2011), while others reported significant clusters of white matter decreases, broadly allocated to bilateral arcuate fasciculus (Ecker, et al., 2012). Alterations in diffusion parameters of frontal and temporal language-related brain regions were reported as well, reflected in reduced fractional anisotropy (FA) values (Barnea-Goraly, et al., 2010; Ke, et al., 2009; Lange, et al., 2010; Lee, et al., 2007) and increased diffusivity (Lange, et al., 2010; Lee, et al., 2007). In the light of the focus of most recent studies, and this thesis, on perisylvian language pathways the following text will summarise our current understanding of this white matter system in autism.

Perisylvian language pathways in autism

To date, the microstructural integrity of perisylvian language pathways in ASDs has been explored in 16 DTI studies, of which 9 used diffusion tractography. All these studies found alterations of perisylvian anatomy ('connectivity') in autism, affecting volumetric tract properties and/or diffusion parameters as indirect measures of white matter integrity. Studies noted abnormalities of the perisylvian language pathways in both left (Ameis, et al., 2011; Barnea-Goraly, et al., 2010; Ben Bashat, et al., 2007; Fletcher, et al., 2010; Jou, et al., 2011a, b; Sahyoun, et al., 2010a,b; Shukla, et al., 2011; Weinstein, et al., 2011) and right hemisphere (Ameis, et al., 2011; Barnea-Goraly, et al., 2010; Cheng, et al., 2010; Cheung, et al., 2009; Jou, et al., 2011b; Kumar, et al., 2010; Lai, et al., 2012; Sahyoun, et al., 2010a; Shukla, et al., 2011) of autistic subjects.

However, methodological constraints limit generalisability of these findings to the broad autism spectrum, since most participants were high-functioning autistic children or adults (e.g. Ameis, et al., 2011; Fletcher, et al., 2010; Sahyoun, et al., 2010a,b), while only one explored perisylvian language pathways in low-functioning children (Lai, et al., 2012). Furthermore, other limitations such as small sample sizes, sample heterogeneity and absence of standardised diagnostic criteria, led to inconsistencies between the findings such as hemispheric differences in the extent of alterations, and direction of abnormalities in diffusion measures. Further replication of these findings is important since structural changes observed are likely to be implicated in language and behavioural deficits in autism. Thus, a recent study of toddlers with autism showed that abnormal structure of the language pathways correlated with delayed language acquisition later in life (Levy, et al., in press). This section will focus on nine diffusion tractography studies that reported diffusion alterations in children, adolescence and adults with ASD.

In very young children (1.5-5.8 years) with ASD increased FA and decreased perpendicular diffusion has been found in the left arcuate fasciculus compared to controls (Weinstein, et al. 2011). The authors explain this FA difference as a consequence of accelerated maturation and brain overgrowth that may occur in ASD brains during early years of life. Further evidence for accelerated maturation of white matter in autism comes from a tract-based spatial statistics (TBSS) analyses (Cheng, et al., 2010; Shukla, et al., 2011), and a high b

value diffusion-weighted imaging study (Ben Bashat, et al., 2007) that reported different developmental curve of bilateral arcuate fasciculus in autism compared to typically developing children. The findings leave open the question on what could cause accelerated maturation in young autistic children - an increase in the number or size of axons, accelerated myelination or reduced synaptic pruning.

In a study of children aged 2.5-9 years, Kumar and colleagues (2010) combined tract based spatial statistics and tractography, and found decreased FA in ASD versus controls in several long-range white matter tracts relevant to communication and socio-emotional functioning, including the right arcuate fasciculus. The left arcuate fasciculus, implicated in majority of other studies, was not found to be affected. Furthermore, fibre volume of the right arcuate fasciculus was positively correlated with stereotypic behaviour, social isolation, and overall autistic triad symptoms, that is, children with higher volume of this tract had more severe autistic symptoms (Kumar, et al., 2010). Similarly, in older children aged 6-12, decreased FA in the right arcuate fasciculus has been found in ASD compared to controls (Poutska, et al. 2011), where FA values were negatively correlated with the severity of ASD symptoms in communication and social interaction but not repetitive behaviour. Older children and adolescents with HFA showed reduced FA in the left (Jou et al., 2011a, 2011b) and right arcuate fasciculus (Sahyoun et al., 2010; Jou et al., 2011a). The only study that investigated low-functioning children with autism reported reduced FA in the left arcuate fasciculus and significant correlation between the affected FA and the activity in the left inferior frontal gyrus for both speech and song conditions (Lai, et al., 2012), further supporting the notion that language dysfunction is associated with the underlying white matter integrity of the perisylvian network.

Alterations in language lateralisation patterns of diffusion measures of adolescents with high-functioning autism were also noticed (Fletcher, et al., 2010; Lo, et al. 2011). Lo, et al. (2011) found that while controls exhibited consistent leftward asymmetry of the long segment of the arcuate fasciculus, there was no such asymmetry present in autistic adolescents. Similarly, a study by Fletcher et al. (2010) showed that the long segment was less lateralised in the autism group. This study found alteration only in the asymmetry of diffusion measures, and not volume. This loss of typical language structural asymmetry points once again to abnormal maturation of brain connections underlying language in autism.

To date, peer-reviewed diffusion tractography studies revealed abnormalities of white matter integrity of perisylvian language pathways, their maturational trajectories and resulting asymmetries. The findings have attracted significant attention since they suggested a link between compromised brain anatomy and deficient language function in autism. This PhD study aims to broaden our understanding of this relationship, by investigating for the first time individual three segments of the arcuate fasciculus, and exploring association between their structural integrity and behavioural language measures in ASD.

6.2 Methods

Subjects

Sixty-one male right-handed adults with ASD (mean age 26.0±7.2) and sixty-one matched neurotypical male controls (mean age 27.7±6.4) aged from 18 to 44 years were recruited by advertisement and subsequently assessed at 1 of 2 collaborating autism research centres in the United Kingdom that make up the Medical Research Council UK Autism Imaging Multicentre Study (MRC AIMS) Consortium: the Institute of Psychiatry, Kings College London; the Autism Research Centre, University of Cambridge. Approximately equal ratios of cases to controls were recruited at each site: London, 35:33, and Cambridge, 26:28 (see Table 6.2.1 for details). Exclusion criteria for all participants included a history of major psychiatric disorder, head injury, genetic disorder associated with autism (e.g., fragile X syndrome and tuberous sclerosis), or any other medical condition affecting brain function (egg, epilepsy). We excluded potential participants who were abusing drugs (including alcohol) and individuals taking antipsychotic medication, mood stabilizers, or benzodiazepines. Also, eight subjects were excluded as outliers based on the quality of imaging data (4 ASD subjects from Cambridge; and 4 controls, 1 from IOP and 3 from Cambridge). All participants with ASD were diagnosed according to International Statistical Classification of Diseases, 10th Revision (ICD-10) research criteria confirmed using the Autism Diagnostic Interview–Revised (ADI-R) (Lord, et al., 1994) to ensure that all participants with ASD met the criteria for childhood autism. All cases of ASD reached ADI-R algorithm cut-off values in the 3 domains of impaired reciprocal social interaction, communication, and repetitive behaviours and stereotyped patterns, although failure to reach cut-off in one of the domains by one point was permitted. Current symptoms were assessed using the Autism Diagnostic Observation Schedule (ADOS) (Lord, et al., 1989) but were not used as inclusion criteria. We also assessed autistic traits in both case and control participants, using the Autism Spectrum Quotient (Baron-Cohen, et al., 2001). Overall intellectual ability was assessed using the Wechsler Abbreviated Scale of Intelligence (WASI) (Wechsler, 1999) All participants fell within the high-functioning range on the spectrum defined by a full-scale IQ higher than 70.. The final sample of 61 individuals who met diagnostic criteria for childhood autism comprised of 24 with a history of delayed language acquisition after 36 months (i.e. subtype of HFA) and 37 individuals who developed phrase speech earlier than 36 months (i.e. subtype of Asperger syndrome). For all subjects, MRI scans were evaluated by an independent clinical neuroradiologists. No gross abnormalities were reported for any of the subjects. All participants gave informed written consent in accordance with ethics approval by the National Research Ethics Committee, Suffolk, England.

Groups	Total	IOP	CAM	AS	HFA
ASD	61	35	26	37	24
Controls	61	33	28	n/a	n/a

Table 6.2.1 Numbers used in the study; IOP - subjects scanned at the Institute of Psychiatry (London); CAM - subjects scanned in Cambridge; AS - Asperger group; HFA - high functioning autism group; n/a - not applicable

DT-MRI acquisition

All participants were scanned using contemporary magnetic resonance imaging (MRI) scanners operating at 3-T (GE Medical Systems HDx, Department of Radiology, University of Cambridge, and GE Medical Systems HDx, Centre for Neuroimaging Sciences, Institute of Psychiatry, Kings College London), with magnetic field gradients (maximum amplitude 40 mT m-1). The body coil was used for RF transmission, and an 8 channel head coil for signal reception, allowing a parallel imaging (ASSET) speed up factor of two. Each volume was acquired using a multi-slice peripherally-gated doubly refocused spin echo EPI sequence, optimized for precise measurement of the diffusion tensor in parenchyma, from 60 contiguous near-axial slice locations with isotropic (2.4 x 2.4 x 2.4 mm^3) voxels. The echo time was 104.5 ms while the effective repetition time varied between subjects in the range 12 and 20 RR intervals. Based on the recommendations by Jones, et al. (2002), the maximum diffusion weighting was 1300 sec/mm^2, and at each slice location, 6 images were acquired with no diffusion gradients applied, together with 32 diffusion-weighted images in which gradient directions were uniformly distributed in space.

DT-MRI processing

Pre-processing and generation of fibre tract data: The diffusion data were analysed using ExploreDTI (Leemans et al., 2009), analysis consisted of (i) correcting for eddy current distortion and subject motion (Leemans and Jones, 2009); (ii) diffusion tensor estimation using a non linear least square method (Jones and Basser, 2004), and (iii) whole brain tractography with a step-size of 0.5 mm, FA thresholds of 0.2 to initiate and continue tracking, and an angle threshold of 35 degrees (Mori and Van Zijl, 2002). To ensure that the observer was blind to hemisphere during virtual dissection of the language pathways and to provide protection against subjective bias, half of the DT-MRI datasets were flipped about the midline.

Visualisation and analysis of fibre tracts: For each individual subject, the high-resolution structural image and the manually segmented structures were registered to the fibre tract data using FLIRT (Jenkinson and Smith, 2001). TrackVis (Wang and Wedeen, 2007) was used for visualising and quantifying fibre tracts. With TrackVis, tract data can be reduced to specific tracts of interest by using a region-of-interest (ROI) selection method (Conturo et al., 1999).

ROI delineation method: A two regions of interest (ROI) approach has been used to dissect the three segments of the perisylvian pathways as described by Catani, et al. (2005). A detailed account of this method can be found in the Methods section of Chapter 2.

Dependent measures

All the measures were calculated using statistics tool in TrackVis. To measure the macro-structural properties of the tracts the number of streamlines within the tract of interest and the number of voxels (volume) through which the fibres of the tract pass were computed. However, to account for individual

variation in brain size, the number of streamlines and volume were co-varied with the total number of streamlines and total brain volume in the analyses. The micro-structural integrity of a tract was quantified by computing the measures of the mean fractional anisotropy (FA), mean diffusivity (MD), and perpendicular diffusivity (Dperp). FA quantifies the directionality of diffusion on a scale from zero (when diffusion is totally random) to one (when water molecules are able to diffuse along one direction only). MD is, as the name suggests, total diffusivity in the tissue, while Dperp measures diffusivity perpendicular to white matter fibres.

A lateralisation index was calculated for each tract based on the number of streamlines, and according to the following formula:

$$\frac{\text{(N. streamlines-left)} - \text{(N. streamlines-right)}}{\text{(N. streamlines-left)} + \text{(N. streamlines-right)}]/2}$$

Positive values of the index indicate a left lateralisation of the variable. Values around zero indicate symmetry between left and right.

Neuropsychological assessments

a) FAS verbal fluency task

Participants were administered the controlled oral word association test (FAS) (Benton, 1968; Benton & Hamsher, 1978) as part of a comprehensive neuropsychological battery. The participants were instructed to say as many words as possible that begin with a letter of the alphabet, excluding proper nouns. The letters F, A, S then were presented in that order. The words produced during a 60-second period for each letter were recorded. The score was the number of words produced. Series of numbers and proper nouns were not scored.

b) Non-word repetition (NWR) task

A test of non-word repetition (NWR) was used to assess phonological short-term memory (Gathercole et al. 1994). In this test, subjects are required to repeat tape-recorded nonsensical words of increasing length and complexity (e.g., “brufid” and “contramponist”). Studies show that individuals with current language impairments, as well as those who had language difficulties in early childhood which later resolved, perform poorly on this test (Gathercole et al. 1994; Bishop et al. 1999). In addition, it has been suggested that performance on the nonword repetition task is the best index of disorder in the KE pedigree known for severe language and speech disturbances (Vargha-Khadem et al. 1998).

c) Verbal IQ

Verbal IQ is part of Wechsler Abbreviated Scale of Intelligence (WASI) and consists of two subtests: vocabulary and similarities. These two subtests compose the verbal scale and yield the Verbal IQ, which is a measure of crystallized abilities. The inter-rater reliability for these tests is: 0.98 (Vocabulary) and 0.99 (Similarities) (Wechsler, 1999).

d) ADOS and ADI-R

Behavioural correlation analyses also were conducted using DTI values and the ADI-R and ADOS sub-scale scores of the ASD group. ADI-R is a semi-structured parental interview that follows DSM-IV criteria for autism (Lord et al., 1994) and evaluates past autistic behaviour, whereas ADOS is a semi-structured interview designed to assess social, communication, play, and stereotyped behaviour and interests at present, and therefore assesses items derived from the communication and social interaction domains (Lord et al., 1989, 1999). DTI measures were correlated with the ADOS sub-scale scores related to current communication impairment, and the ADI-R sub-scale scores related to past communication deficits. The ADI-R, and ADOS items that were used in the analyses are shown in Table 6.2.2.

Instrument	Items	Behaviour
ADI-R	Q33	stereotyped utterances and delayed echolalia
	Q34	social verbalization/chat
	Q35	reciprocal conversation
	Q36	inappropriate questions or statements
	Q37	pronominal reversal
	Q38	neologisms / idiosyncratic language
	Total Q	Total score (Q33 + Q34 + Q35 + Q36 + Q37 + Q38)
ADOS	A-4	stereotyped and/or idiosyncratic use of words, phrases
	A-8	conversation
	Total A	Total score (A4 + A8)

Table 6.2.2 ADI-R and ADOS items used in a correlation analysis between behaviour variation and perisylvian language network anatomy

e) Autism Spectrum Quotient (AQ) test

The AQ test is a brief, self-administered instrument for measuring the degree to which an adult with normal intelligence has the traits associated with the autistic spectrum. Individuals score in the range of 0-50, with higher scores defining autistic traits. AS and HFA groups usually score 32+, compared to controls whose score is lower by half (Baron-Cohen, et al., 2011). The AQ sub-scores include: Communication, Social, Imagination, Local Details, and Attention Switching. In this study two domains were used in the correlational analysis: Communication and Social domain, as they might be related to the underlying functions of the perisylvian language network.

f) Empathy Quotient (EQ) test

Empathy Quotient (EQ) test is a self-report questionnaire, for use with adults of normal intelligence when measuring empathy, essential for social functions. It contains 40 empathy items and 20 filler/control items, on which individuals can score in the range of 0-80, with the lower scores characteristic for autism (scores lower than 30) (Baron-Cohen and Wheelwright, 2004).

Statistical analysis

Statistical comparisons of the demographic and behavioural data were performed using SPSS 16.0 software for Apple Mac (SPSS Inc, Chicago, IL). For all analyses, the level of statistical significance was defined as $p < .05$ (two-tailed). Overall group differences in age, IQ data, and behavioural performances were calculated using an independent samples t-test.

Tractography

The tractography data were first subject to a two-tailed 1-Sample K-S test to verify normal distribution. Because normal distribution was found for all the measures, parametric tests could be adopted to examine group differences. Differences between ASD and healthy controls were assessed using ANOVA and regression models. Analysis comparing the three groups, AS, HFA, and control groups, was done using an ANOVA test and applying Bonferroni correction for multiple comparisons. Logistic regression was used to compare the autism group (AS and HFA together) and controls, and co-vary for centre, age (for FA measures), total number of streamlines or total brain volume (for the measures of number of streamlines and volume of specific tract).

Behavioural

The relationship between the specific anatomical differences in perisylvian language pathways and domains of symptom severity (ADI-R and ADOS) was investigated within ASD group using ANOVA analysis. Symptom severity measures included 6 items of the ADI-R measuring past communication symptoms at ages 4 to 5 years, and their total score, and 2 items of the ADOS assessment (communication scores) of current symptom severity, and their total score (see Table 6.2.2).Furthermore, relationships between behavioural measures and anatomy were explored using Pearson correlation coefficients across the whole sample and within the subtypes. Behavioural tests included the FAS verbal fluency task, NWR task, verbal IQ, AQ and EQ tests. The goal was to elucidate the relationship between structural connectivity and dimensions of behaviour across the whole sample..

6.3 Results

Participants Demographics

There were no significant differences (Independent samples t-test, 2-tailed) between the ASD and control groups with regard to age or full scale IQ (see Table 6.3.1).

As expected ASD subjects performed significantly worse than controls on the FAS verbal fluency (t=2.015, p=0.046), AQ (t=-12.049, p=.000) and EQ (t=10.012, p=.000) tests, together with AQ test sub-domains, Communication domain (t=-11.679, p=.000) and Social domain (t=-9.993, p=.000). Other behavioural tests did not differ significantly between the groups (see group statistics in Table 6.3.1 and t-test results in Table 6.3.2, Appendix B).

Variable	Controls (mean± STD)	ASD group (mean± STD)	t	Sig. (2-tailed)
Age	27.67 ± 6.4	26.05 ± 7.2	1.332	0.185
Full IQ	114.54 ± 11.6	111.57 ± 12.2	1.374	0.172

Table 6.3.1 No significant difference was found between ASD and control group with regard to age and full IQ

Splitting the autism group

There were no significant differences in behavioural measures between HFA and AS group (Table 6.3.3, Appendix B).

Tractography reconstructions

Tractography reconstructions of the three segments in each hemisphere were performed (see Fig 6.3.1).

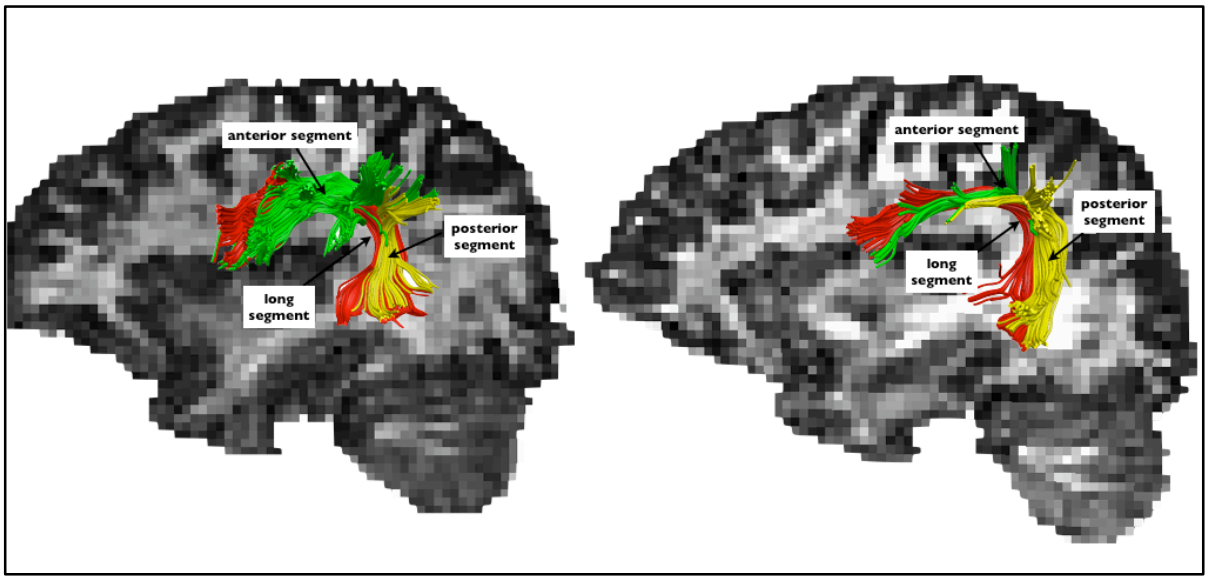

Fig 6.3.1 Tractography reconstructions of the long (in red), anterior (in green) and posterior (in yellow) segments of the perisylvian language network from a typical healthy subject (on the left) and an ASD subject (on the right).

Between-group differences in DTI parameters

Dimensional approach - tract-specific differences

People with ASD had significantly higher mean diffusivity (MD) (p=0.002; p=0.003) and perpendicular diffusivity (Dperp) (p=0.003; p=0.003) values than controls in the long left (Table 6.3.5, Appendix B) and anterior left segment (Table 6.3.6, Appendix B), and significantly higher MD in the posterior segment of both hemispheres (p=0.007 for posterior left and p=0.005 for posterior right segment) (Table 6.3.7 and Table 6.3.10, Appendix B). In contrast ASD individuals had a significantly lower fractional anisotropy (FA) (p=0.025), number of streamlines (p=0.031), and volume (p=0.025) in the anterior left segment compared to controls (Table 6.3.6, Appendix B), and number of streamlines of the left long segment (p=0.039) (Table 6.3.5, Appendix B) There were no significant between group differences in the right anterior and right long segment (Table 6.3.8 and Table 6.3.9, Appendix B). The most affected diffusion index in the ASD population was MD, which was significantly decreased in all the tracts implicated. Fig 6.3.2 provides graphical summary of the findings. Furthermore, no significant differences were found for the lateralisation indices (based on the number of streamlines) of the perisylvian tracts between ASD group and controls.

Hemisphere	Tract	FA	MD	Dperp	NoSt	Vol
Left	Long	↓*	↑***	↑***	↓*	n.s.
	Anterior	↓*	↑***	↑***	↓*	↓*
	Posterior	n.s.	↑***	n.s.	n.s.	n.s.
Right	Long	n.s.	n.s.	n.s.	n.s.	n.s.
	Anterior	n.s.	n.s.	n.s.	n.s.	n.s.
	Posterior	n.s.	↑***	↑*	n.s.	n.s.

Fig 6.3.2 Overview of the significant differences and direction of differences found in the ASD group compared to controls, in FA - fractional anisotropy, MD - mean diffusivity, Dperp - perpendicular diffusivity, NoSt - number of streamlines, Vol - volume; n.s.- not significant, * significant at $p < 0.05$; *** significant at $p < 0.01$

Categorical approach - splitting the ASD group

There were no significant differences in the anatomy of the arcuate fasciculus measured by diffusion tractography between HFA and AS group. Hence, only dimensional approach was used, and not categorical (HFA versus controls, and AS versus controls).

Relating behavioural variation to anatomy of perisylvian language pathways

Association with past language use in autism

We found that within people with autism, there was a highly significant relationship ($r= -.359$, $p=0.009$) between number of streamlines in the anterior left segment and severity of stereotyped utterances and delayed echolalia in childhood, ages 4-5 years (as measured by the ADI-R Q33) (see Fig 6.3.3). We performed ANOVA analysis (with Bonferroni correction) and obtained significant differences in mean values of the number of streamlines between the groups of different symptom severity. The more severe the symptoms were during childhood, the less was the number of streamlines present in adulthood.

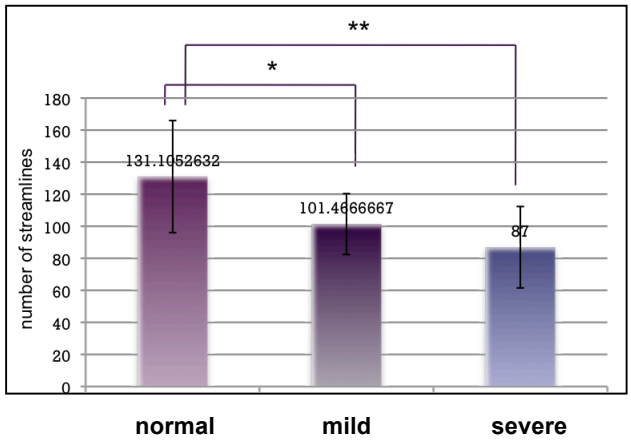

Fig 6.3.3 Histogram of the mean number of streamlines (±95% confidence interval) in the left anterior segment for the three subgroups of ASD patients divided according to the severity of stereotyped utterances and delayed echolalia (for ADI-R question 33, score 0 corresponds to normal, 1 to mild symptoms, 2 to severe symptoms). (* p=0.035 between normal and mild, after Bonferroni correction; ** p=0.027 between normal and severe, after Bonferroni correction).

Relationship with present behaviour in autism

Using ANOVA analysis, no significant relationship was found between present language deficits measured by ADOS-R and the anatomy of the perisylvian language pathways within people with autism.

Relationship with present behaviour across the whole sample

The relationships between regional anatomic abnormalities and dimensions of behaviour across the whole sample were explored using Pearson correlation coefficients. Within the whole sample there were significant positive and negative correlations (in the right direction) between white matter language integrity (MD and Dperp values) of the left long and left anterior and MD values of bilateral posterior segment and Communication and Social scores from Autism Spectrum Quotient (AQ), and Empathy Quotient (EQ) scores. AQ Communication scores were positively correlated diffusion measures of the left perisylvian tracts: long (MD r=.200, p=.030; Dperp r=.219, p=.017), anterior (MD r=.224, p=.014; Dperp r=.213, p=.020) and posterior (MD r=.211, p=.021) segments (see Appendix B Table 6.3.11). AQ Social scores were positively correlated with MD values of the anterior (r=.182, p=.048) and posterior (r=.189, p=.038) segment in the left hemisphere (see Appendix B Table 6.3.12). A significant negative correlation was observed between the empathy scores on the EQ test and diffusion measures of the left long (MD r=-.221, p=.017; Dperp r=-.189, p=.041), left anterior (MD r=-.228, p=.013; Dperp r=-.220, p=.017), and bilateral posterior segment (MD r_L=-.208, p=.023; MD r_R=-.185, p=.045). Data plots for each correlation are shown in Appendix B, Figures 6.3.14-21. No significant correlations were found for the scores on the FAS verbal fluency and NWR tasks, or verbal IQ. However, the results across the whole sample were not replicated within each sub-sample, and hence it is likely that the differences between the cases (autistic individuals) and controls are driving these correlations towards significance. Data plots for each affected regions are shown in Appendix B, Figures 6.3.14-21.

6.4 Discussion

This study is the first large-scale multi-centre MRI study to investigate perisylvian language pathways in a well-characterised sample of men meeting the ADI-R research diagnostic criteria for autism. Significant alterations of the left perisylvian white matter network including long, anterior and posterior segments of the arcuate fasciculus were found, some of which related to the severity of language deficits.

6.4.1 Analysis of the perisylvian language pathways in ASD: main findings

Tract-specific differences: left frontal perisylvian connections most affected in ASD

This study found significant differences in the language network that mostly affected left hemisphere frontal lobe connections (long and anterior segment) linking inferior frontal gyrus to parietal and temporal regions. These brain areas have previously been implicated in language (Groen et al, 2008) and mirror neuron system (Dapretto et al, 2006; Oberman et al, 2005; Uddin et al, 2008) dysfunction in autism.

Adults with ASD had significantly higher mean diffusivity (MD) and perpendicular diffusivity (Dperp) than controls in the left long and left anterior segment. In contrast, ASD individuals exhibited significantly lower fractional anisotropy (FA), number of streamlines, and volume in the left anterior segment compared to controls, although the mentioned differences did not stand the test of multiple comparisons, and should be interpreted as a trend only. Nevertheless, the results reveal that left frontal perisylvian connections are the ones most affected in ASD.

Previous diffusion studies reported diffusion differences in the left long segment (Ben Bashat, et al., 2007; Fletcher, et al., 2010; Jou et al., 2011a, 2011b; Lai, et al., 2012; Weinstein, et al., 2011;) and left anterior segment (Barnea-Goraly, et al., 2010; Ben Bashat, et al., 2007) in autistic children and adolescents. It was suggested that these differences reflect abnormal and accelerated maturation of the left frontal lobe connections during childhood (Ben Bashat, et al., 2007). However, most of these studies were limited in terms of sample size and did not investigate structural connectivity in adults with autism. Ours is the first large tractography study to confirm that structural alterations of the frontal lobe connections are present also in adults with autism, suggesting that anatomical differences in childhood persist into adulthood. Functional alteration of these frontal lobe connections were previously confirmed in functional MRI studies of autistic adults. Differences in the microstructure of the left long segment may be the structural correlate of decreased functional connectivity between Wernicke's and Broca's areas during language comprehension in autism (Just et al., 2004; Knaus et al., 2008). On the other hand, anatomical alterations of the anterior segment of the left hemisphere might represent a neural substrate for decreased fronto-parietal functional connectivity during language and social tasks (Just et al., 2007; Kana et al., 2006; Koshino et al., 2005; Lombardo, et al., 2010) observed in autism.

Besides differences in frontal lobe connections, we observed significant differences in the temporo-parietal connections bilaterally in adults with autism. This was reflected in significantly higher MD bilaterally, and significantly higher Dperp value in the right hemisphere of posterior segment. Observed alterations of the temporal connections (long and posterior) might be a consequence of atypical maturation observed in posterior temporal brain regions in autism (Shih, et al., 2011). These temporal abnormalities are likely to lead to language-related learning deficits in autism during development (Kuhl et al. 2005; Scott-Van Zeeland, et al., 2010b), since recent evidence shows that the posterior segment is involved in language-related learning through syllable discrimination and identification (Parker, et al., 2005).

Based on the 'parallel pathway model' and possible functional correlates of specific perisylvian language tracts some authors have argued that indirect pathway will be more affected than direct pathway in autism (Fletcher, et al., 2010). This suggestion is based on prior investigations of aphasic patients that reported that lesions of the long direct segment result in Wernicke's "conduction" aphasia characterised by impaired passive repetition but relatively preserved spontaneous speech and language comprehension, whereas lesions of the indirect pathways (anterior and posterior) result in transcortical motor aphasias characterised by relatively intact passive repetition but impaired spontaneous speech and/or comprehension (Catani et al., 2005). Hence, given the frequency of immediate echolalia during early childhood the indirect pathways might be more impaired in verbal individuals with autism than the long direct pathway (Fletcher, et al., 2010). However, our findings do not support that suggestion as we found both long and anterior pathways of the left hemisphere significantly different in autism.

Furthermore, our study failed to find differences in lateralisation patterns (based on the number of streamlines as a proxy measure of volume) of the perisylvian language network. This finding might seem somewhat unexpected if we have in mind that recent structural MRI studies point to the loss of left asymmetry in the volume of both frontal and temporal language regions, more specifically Broca's area (De Fosse et al., 2004; Herbert et al., 2002; Tager-Flusberg and Joseph, 2003), planum temporale and Heschl's gyrus area (Rojas et al., 2002, 2005) in autism. However, when exploring the asymmetry of perisylvian white matter connections, this study found no significant differences among the two groups. This is in line with the findings by Fletcher et al. (2010) who found similar volumetric asymmetry of the long segment in individuals with high-functioning autism compared to controls.

Using categorical approach to autism, previous voxel-based morphometry studies reported differences in grey and white matter of language-related brain regions between autism and Asperger syndrome (AS) (McAlonan, et al., 2005, 2009). However, this approach is limited in being unable to localise the differences to specific white matter tracts and unable to examine the underlying white matter integrity. Our study failed to support that there are significant anatomical differences between the high-functioning autism (HFA) compared to AS. We observed no significant differences in the anatomy of the perisylvian language network between these two groups. Hence, our findings suggest that delay in acquisition of language in ASD is not

associated with anatomical variation in the perisylvian network, but some aspect of overall language skill is. This findings gives further support to the recent transition from categorical to dimensional approach to autism.

Differences among DTI-extracted parameters

The neuropathology of the perisylvian language pathways in autism was more evident in diffusion measures - implicating white matter microstructure, than in volumetric measures (volume and number of streamlines). The diffusion measure most affected in the ASD population was MD, which was significantly decreased in all the tracts implicated. This is in line with the studies noting that MD is a more sensitive marker of neuropathology compared to FA or volumetric measures in autism (Ameis, et al., 2011; Fletcher, et al., 2010; Nagae, et al., 2012), primary progressive aphasia (Galantucci, et al., 2011) and Alzheimer's disease (Acosta-Cabronero, et al., 2010). The potential biological implications will be discussed in the section 6.4.4.

6.4.2 Relating behavioural variation to anatomy of perisylvian language pathways

The question remains as to how these organic brain deficits map onto what is known about the language functioning in adults with autism. Hence, the relationships between regional anatomic abnormalities and domains of symptom and behaviour severity were explored. White matter integrity of perisylvian pathways has previously been correlated with language deficits in childhood autism (Levy, et al., in press), while this study gave the first evidence of this association in autistic adults, and showed that differences in childhood persist into adulthood and continue to be associated with clinical symptoms.

Relationship with past language use in autism

Within people with ASD, there was a highly significant relationship between the number of streamlines in the anterior left segment and severity of stereotyped utterances and delayed echolalia in childhood. The more severe the symptoms were during childhood, the less was the number of streamlines present in adulthood. Echolalia is explained as a failure of normal imitation of speech, and represents a normal phenomenon in the learning of language in infancy (Lecours, et al., 1983). In general, imitation was found to be of crucial value for development of language, but also the normal development of pretend play and socially insightful behaviour (McEwen, et al., 2007) that are typically impaired in autism. Some authors suggested that echolalia represents intactness of primary language areas in the frontal and temporal lobes, with syntax unimpaired but disconnected from control (Hadano, et al., 1998; Mendez, 2002). I could speculate that this disconnection might be partly explained by the reduced connectivity of the left anterior segment in autistic children, possibly due to aberrant maturation, which persists into adulthood. Another link between the anterior segment and the impaired imitation comes from recent studies of mirror neuron system. The neural basis for vocal learning and imitation is though to involve mirror neuron system in humans, which if disrupted is likely to result in echolalia. This systems contains also the fibres of fronto-parietal (anterior

segment) connections (Aboitiz, 2012), which could thus be the neural basis for echolalia in autism. However, many of the mirror neuron studies are still controversial, and need further replication. Finally, the question remains of how reliable is it to study the association between past language deficits and the anatomy in adulthood. Furthermore, we found no association between anatomy and the severity of language deficits in adulthood, as measured by ADOS. Thus, it is possible that individuals with ASD partly overcome these language deficits by compensation mechanisms or simple developmental factors, as recently reported in several behavioural studies (Eisenmajer, et al., 1996; Gilchrist, et al., 2001; Howlin, 2003).

Relationship with present behaviour across the whole sample

This study found significant relationship between structural connectivity and dimensions of behaviour across the whole sample after performing correlation analysis between anatomy and measures of communication and social domains of Autism Quotient (AQ) test and empathy of Empathy Quotient (EQ) test. The more severe, 'autistic', the deficits in communication and social domains were, the higher MD and Dperp were in the three segments of the left perisylvian network. Furthermore, higher MD and Dperp of the left frontal lobe connections (long and anterior segment) and higher MD of the posterior segment bilaterally, were associated with more severe deficits in empathy across the whole sample. However, the results across the whole sample were not replicated within each sub-sample, and hence the differences between the cases and controls were probably driving these correlations towards significance.

6.4.3 Limitations

We have included individuals fulfiling 'gold standard' diagnostic criteria (i.e., who were above threshold in both the ADI-R and ADOS-R) and who did not differ in gender, age or overall intellectual functioning from controls. Both ADI-R and ADOS scores were chosen as exclusion criteria because current symptoms assessed in adult samples can often be masked by coping strategies developed as the person ages and can also be alleviated by treatments and/or interventions (e.g., social skills training), whereas the accuracy of ADI-R may sometimes be uncertain given the reliance on retrospective parent report. Using both ADI-R and ADOS-R lent confidence to the findings of between-group differences. Nevertheless, there are several limitations to this study. Firstly, due to the benefit of decreasing variability of the sample and thus increasing statistical power, only verbal males with normal intelligence were included. Hence, it is not known to what extent would these findings generalise to females, cognitively lower functioning and non-verbal individuals with autism, or children. This generalisation is especially problematic when considering children with autism, since age was revealed to be an important factor influencing autistic neuropathology (Herbert, et al., 2003; McAlonan, et al., 2002). It is necessary to detect these differences during development of brain structure and function, and not merely as end-products of brain systems' neuropathology in adulthood. In order to study the brain anatomy in autism throughout the lifespan, longitudinal or large cross sectional designs with different age cohorts should be employed. Secondly, a multicentre design was used for MRI data acquisition to overcome single-site recruitment limitations, but similarly poses a methodological issue affecting the reliability of findings. However, inter-site effects were accounted for in the statistical model, by using different

centres as covariates. Therefore, the detected between-group differences cannot be fully explained by these limitations. Lastly, the methodological limitations of DTI tractography need to be acknowledged. Tractography cannot visualise axons directly, and hence represents merely an indirect measure of white matter tracts. Furthermore, methodological considerations potentially impacting measurement of FA, MD, Dperp (partial volume effects, signal-to-noise ratio of the data) need to be acknowledged (see chapter 1 for more details on DTI limitations). Additional imaging techniques such as magnetisation transfer imaging, MR spectroscopy, and relaxation time measurements may help to increase the specificity of our FA findings (Kubicki et al., 2005).

6.4.4 Implications

As the autism field strives for sensitive and specific biomarkers of ASD, these findings offer hope for future research, suggesting a possibility of non-invasive, brain-based screening methods that could detect anatomical differences possibly even prior to behavioural emergence. However, the question remains whether these changes in white matter structure are a direct cause of the disorder or alternatively, a secondary consequence of abnormal brain function and the result of living one's whole life with autism. Our findings of significant relationship between altered anatomy in adulthood and language deficits relevant to earlier developmental stages, suggest that these perisylvian white matter differences are likely present in the brain of young autistic children. Hence, it is possible that altered structural connectivity of the perisylvian language network is one of the core features of autistic brain. These results place further importance on exploring how structural changes co-evolve with the language deficits observed in autism. A longitudinal study is necessary in order to provide a detailed profile of atypical development, sub-categorisation of the behavioural phenotype, and (potentially) prediction of treatment response (e.g. to language training). For example, bearing in mind the continued maturation of the arcuate fasciculus in the healthy population (Brauer, et al., 2011) it may be possible to explore whether specific interventions that improve language functioning in individuals with autism do so by modulating the development of white matter microstructure.

Potential neurobiological implications

The changes in microstructure of the left perisylvian language pathways suggested by our findings could be due to a number of different processes. Since autism is a developmental disorder with onset in early childhood, the micro-structural changes may be a persisting manifestation of a primary abnormality of early development of the arcuate fasciculus (Fletcher, 2010). Alternatively, the microstructural changes in white matter could be secondary to abnormal development of cortical minicolumns (Casanova et al., 2009) and/or cortical dysgenesis of the frontal and temporal lobes (Bailey et al., 1998). It is also possible that the observed changes are the *result* of abnormal language functioning rather than it's cause (Paus et al., 1999).

It is important to note the neuroanatomical implications and discuss which neurobiological changes might give rise to the present tractography findings. It is known that the relative sensitivity of the various diffusion parameters in identifying abnormalities is dependent upon the underlying pathology (Kumar, et al., 2009). For example, if there is a change in diffusion in all directions, such as in stroke or cell death, MD is likely to be a very sensitive measure as it represents the average of diffusivities along three main directions. We found that MD was significantly different – but so were other parameters such as also Dperp . Hence our results most likely arise from a complex mixture of microstructural changes. Increased MD found in this study may reflect demyelination, axonal damage (Basser, 1995), or loss of white matter coherence (Basser and Pierpaoli, 1996; Werring et al., 2000a), while increased Dperp is by some considered a sensitive marker for demyelination (Song et al., 2005) but this is questionable (Wheeler-Kingshott and Cercignani, 2009). Hence, the differences in the frontal lobe connections of the left hemisphere and bilateral temporo-parietal segment in ASD likely reflect the mixture of underlying biological changes such as demyelination, axonal damage (e.g. decrease in the number of axons, an increase in intra-axonal space etc).

6.4.5 Conclusions

Our findings show that diffusion tractography is sensitive to anatomical difference in the language pathways of normal intelligence adults with ASD. These differences are mainly localised in the left perisylvian pathways and relate to the severity of stereotyped utterances and delayed echolalia in childhood. Abnormal development of the left frontal perisylvian connections may partially explain some of the abnormal imitative behaviours and impaired communication typically found in ASD. In conclusion this is the first in vivo study to identify localised abnormalities in the left perisylvian language pathways of people with ASD and to demonstrate a significant relationship between abnormalities in white matter integrity and the severity of language deficits.

Chapter 7

Final Remarks

The intention of this PhD project is to bring significant experimental and theoretical extension of knowledge to our understanding of the anatomy of the perisylvian language network in the living human brain. The presented studies have tried to determine the role that age, genes and environment play upon the anatomy of the perisylvian language pathways in healthy population and how impaired language development affects the anatomy of these perisylvian connections.

The results presented herein have showed that the perisylvian language pathways exhibit distinct maturational patterns and are under different extent of genetic control. The frontal lobe connections were shown to lateralise relatively early in life, which is in line with imaging studies that observed early asymmetric organisation of the arcuate fasciculus (Dubois, et al., 2009; Eluvathingal, et al., 2007; Lebel and Beaulieu, 2009). Prior to adolescence the left long segment is larger than the right, and the right anterior segment is larger than its left counterpart, and this arrangement remains stable throughout adulthood. Early structural asymmetries of the long and anterior segments suggest that structural organisation and maturation of this network might underlie the brain's functional lateralisation. Support for this notion comes from a recent study reporting that early frontal maturation is sufficient to sustain language functional activity (Leroy, et al., 2011). Furthermore, functional studies showed this area to be active in infants when listening to speech (Dehaene-Lambertz et al., 2006; Bristow et al., 2009). On the other hand, temporo-parietal connections exhibit a dynamic pattern of lateralisation, and continue to lateralise during adolescence and early adulthood due to the loss of white matter connections in the right hemisphere. The question remains whether this maturational pattern of the posterior segment is linked to children's stronger reliance on the right hemisphere, reflected in a more rightward functional lateralisation during language processing as compared to adults (Brauer and Friederici, 2007), which ceases in later years due to the loss of connections in the right hemisphere.

Knowing that experience changes white matter (Fields, 2008), and affects dendritic branching of neurones and the numbers of synaptic connections (Toga et al., 2006) it might be expected that the effects of environment will play a bigger role on the temporo-parietal connections (posterior indirect segment) that undergo a more dynamic maturational pattern, compared to the frontal lobe tracts, long direct and anterior indirect segment, that show early development. Our results suggest this to be the case. Genetic analysis revealed that temporo-parietal dynamic maturational pattern is mostly driven by specific environmental factors that twins do not share. Conversely, frontal lobe connections that lateralise very early in life exhibit a higher degree of familial control (genes and shared environment) in adulthood. This is also in agreement with studies that noted a lower degree of genetic contribution to those brain structures that appear later in cerebral development (Brun, et al., 2008; Lohmann, et al., 1999). However, we need to be aware that genes and environment are not independent of each other, and that genetic factors can drive the exposure to certain environmental settings and relevant experiences. Thus we need to interpret these findings with some caution. Also we need to be aware that heritability changes with age, with regions associated with complex cognitive processes such as language being more heritable in adolescents than children (Lenroot, 2009), and the same being true for cognitive functions such as prosocial behaviour, IQ and general cognitive ability *g* (Plomin et al., 1997). It is thus possible that strong genetic effects are more likely present during later years for the posterior indirect segment.

Maturational and heritability differences between posterior indirect segment and the frontal connections, long and anterior segment, raise questions whether posterior segment is functionally different from the other two. Previous study found that differences in maturation of the left language connections were associated with differences in cognitive abilities (Lebel and Beaulieu, 2009). The observation that developmental changes of the posterior segment are most prominent during adolescence could be related to increasingly complex functional requirements. This is in line with the findings suggesting that temporo-parietal connections are important for higher order cognitive functions that continue to develop throughout adolescence and adulthood, including theory of mind (Apperly et al., 2004; Njomboro, et al., 2008; Samson, et al., 2004; Saxe and Kanwisher, 2003), control of intention to speak (Carota et al., 2010; Desmurget et al., 2009), speech self-awareness (Jardri, et al., 2007), word semantics and conceptual semantics (Friederici, et al., 2010), verbal working memory (Jacquemot and Scott, 2006), and so on. It is likely that posterior segment acts as a neural substrate for higher mental processes that involve both language and social cognition. Hence, our results are in line with a hypothesis that long direct segment supports early crucial stages of language acquisition, while indirect pathways become more relevant for complex processing during later stages of language development (Perani et al., 2011). Our findings also lend support to the view that different aspects of language skills, being supported by different perisylvian segments, are under different hereditary mechanisms (Stromswold, 2001). Furthermore, our study supports the view that later developing white matter structures (i.e. posterior segment) are more vulnerable to environmental stressors (Kochunov, et al., 2012; Rosenzweig, et al., 2012). This is because later developing myelin is exceptionally vulnerable to subtle metabolic and oxidative abnormalities during developmental and degenerative phases (Bartzokis, 2011).

The understanding of developmental patterns has vital implications for neurodevelopmental disorders that manifest with language pathology. The application of DTI tractography in the final study offered insights on how language pathology affects the anatomy of perisylvian language networks in adults with autism spectrum disorders (ASD). Significant differences in the left perisylvian language connections were found in ASD, including long, anterior and posterior segments. Previously left arcuate fasciculus was implicated in children with autism (Jou et al., 2011a, 2011b; Lai, et al., 2012; Weinstein, et al., 2011), but our study is the first to show that these anatomical differences persist from childhood into adulthood. Furthermore, the observed structural differences in anterior segment connections in adulthood were associated with the severity of the past language use in autism, suggesting that these anatomical abnormalities might be present already in childhood, as a result of abnormal maturation. It is possible that aberrant perisylvian language connections represent one of the key features of autistic brain. Further, our study suggests that delay in acquisition of language in ASD is not associated with anatomical variation in the perisylvian network but some aspect of overall language skill is, since we found no structural differences between high-functioning autism and Asperger syndrome. Hence, these findings give further support to dimensional approach for classifying ASD. Importantly, autism spectrum disorder, which is a highly heritable neurodevelopmental disorder, exhibited most abnormalities in those tracts that were found to be most heritable in the twins study - frontal lobe connections. Furthermore, the diffusion measure most affected in autism was mean diffusivity, which was the measure under the highest genetic control in the twin study. Our results therefore implicate those structures and measures with the highest heritability estimates as potential sensitive biomarkers for autism. The findings lend support to the already reported notion that mean diffusivity is a more sensitive marker of neuropathology compared to fractional anisotropy or volumetric measures (Nagae, et al., 2012; Galantucci, et al., 2011). Results highlight the relevance of frontal perisylvian network in the future genetic linkage and association studies, and their hunt for genes influencing language-related brain structure and function. The relevant susceptibility genes, once identified, could be informative for understanding the evolution of social cognition and how this relates to language origins. Also, these findings offer hope for future research in specific biomarkers in autism spectrum disorder, suggesting a possibility of non-invasive, brain-based screening methods that could detect anatomical differences possibly even prior to behavioural emergence.

In conclusion, it is hoped that the research summarised in this PhD thesis will contribute to better understanding of the anatomy of the perisylvian language pathways and the complex relationship between language impairments in psychiatric disorders and their neural correlates, facilitating better diagnostic and treatment schedules for affected individuals. However, future studies should explore the ventral pathways which are also important for language and social cognition in humans. However, as David Hubel notes, to know the connections of a structure within the brain is a quite different from understanding the structure's physiology (Hubel, 1995). How individual neurons work, how they generate electrical signals and convey information to other cells, and how they interact to produce a system capable of supporting language processing remains unanswered. Understanding the structure and function of a trait as complicated as language requires a synthesis of future multidisciplinary research from neuroscience, genetics, linguistics, psychology and developmental biology. This thesis is plainly just one piece of the research puzzle, but it provides an exciting step forward in investigations of language in the human brain.

Appendix A

Chapter 4.
Tables of appendix A:

Table 4.2.1 Equality of means and variances within zygosity groups (paired t-test for means, Levene's test for variances) and across zygosity (independent t-test). p value is significant at p< .05

		AF NO_TRACTS			AF VOLUME			AF FA			AF MD		
		Equality of Means (p value)		Equality of variance (p value)	Equality of Means (p value)		Equality of variance (p value)	Equality of Means (p value)		Equality of variance (p value)	Equality of Means (p value)		Equality of variance (p value)
Long Left	MZ1 + MZ2	0.841	MZ	0.275	MZ1 + MZ2	0.780	MZ	0.319	MZ1 + MZ2	0.769	MZ	0.496	MZ1 + MZ2	0.152	MZ	0.436
	DZ1 + DZ2	0.680	DZ	0.894	DZ1 + DZ2	0.624	DZ	0.940	DZ1 + DZ2	0.279	DZ	0.866	DZ1 + DZ2	0.123	DZ	0.432
	all MZ + all DZ	0.780	all MZ + all DZ	0.604	all MZ + all DZ	0.363	all MZ + all DZ	0.176	all MZ + all DZ	0.277	all MZ + all DZ	0.891	all MZ + all DZ	0.126	all MZ + all DZ	0.051
Long Right	MZ1 + MZ2	0.838	MZ	0.517	MZ1 + MZ2	0.588	MZ	0.201	MZ1 + MZ2	0.929	MZ	0.309	MZ1 + MZ2	0.696	MZ	0.266
	DZ1 + DZ2	0.924	DZ	0.594	DZ1 + DZ2	0.904	DZ	0.522	DZ1 + DZ2	0.487	DZ	0.943	DZ1 + DZ2	0.817	DZ	0.953
	all MZ + all DZ	0.132	all MZ + all DZ	0.998	all MZ + all DZ	0.160	all MZ + all DZ	0.617	all MZ + all DZ	0.581	all MZ + all DZ	0.244	all MZ + all DZ	0.346	all MZ + all DZ	0.522
Anterior Left	MZ1 + MZ2	0.984	MZ	0.371	MZ1 + MZ2	0.710	MZ	0.095	MZ1 + MZ2	0.090	MZ	0.482	MZ1 + MZ2	0.151	MZ	0.432
	DZ1 + DZ2	0.324	DZ	0.680	DZ1 + DZ2	0.392	DZ	0.438	DZ1 + DZ2	0.478	DZ	0.149	DZ1 + DZ2	0.390	DZ	0.121
	all MZ + all DZ	0.056	all MZ + all DZ	0.848	all MZ + all DZ	0.069	all MZ + all DZ	0.164	all MZ + all DZ	0.194	all MZ + all DZ	0.603	all MZ + all DZ	0.582	all MZ + all DZ	0.391
Anterior Right	MZ1 + MZ2	0.220	MZ	0.257	MZ1 + MZ2	0.251	MZ	0.301	MZ1 + MZ2	0.512	MZ	0.270	MZ1 + MZ2	0.078	MZ	0.899
	DZ1 + DZ2	0.726	DZ	0.319	DZ1 + DZ2	0.514	DZ	0.693	DZ1 + DZ2	0.560	DZ	0.783	DZ1 + DZ2	0.510	DZ	0.384
	all MZ + all DZ	0.110	all MZ + all DZ	0.828	all MZ + all DZ	0.079	all MZ + all DZ	0.264	all MZ + all DZ	0.414	all MZ + all DZ	0.96	all MZ + all DZ	0.642	all MZ + all DZ	0.105
Posterior Left	MZ1 + MZ2	0.863	MZ	0.109	MZ1 + MZ2	0.272	MZ	0.472	MZ1 + MZ2	0.613	MZ	0.115	MZ1 + MZ2	0.177	MZ	0.826
	DZ1 + DZ2	0.878	DZ	0.861	DZ1 + DZ2	0.864	DZ	0.789	DZ1 + DZ2	0.862	DZ	0.927	DZ1 + DZ2	0.223	DZ	0.255
	all MZ + all DZ	0.063	all MZ + all DZ	0.387	all MZ + all DZ	0.088	all MZ + all DZ	0.601	all MZ + all DZ	0.053	all MZ + all DZ	0.289	all MZ + all DZ	0.135	all MZ + all DZ	0.201
Posterior Right	MZ1 + MZ2	0.870	MZ	0.072	MZ1 + MZ2	0.492	MZ	0.070	MZ1 + MZ2	0.515	MZ	0.735	MZ1 + MZ2	0.091	MZ	0.520
	DZ1 + DZ2	0.927	DZ	0.761	DZ1 + DZ2	0.811	DZ	0.231	DZ1 + DZ2	0.052	DZ	0.975	DZ1 + DZ2	0.882	DZ	0.224
	all MZ + all DZ	0.171	all MZ + all DZ	0.055	all MZ + all DZ	0.088	all MZ + all DZ	0.063	all MZ + all DZ	0.117	all MZ + all DZ	551	all MZ + all DZ	0.850	all MZ + all DZ	0.239

Table 4.3.1 Intraclass correlation coefficients, ACE estimates (with 95% Confidence Intervals), and SEM model fitting estimates for the Number of Streamlines of the three segments of AF in MZ and DZ twins

AF			Intraclass Correlations		ACE Model			Conclusions of Best Fit		
Tract	Hemisphere	Variable	MZ	DZ	a2	c2	e2		chi-sq / df=1	p
LONG SEGMENT	Left	Number of Tracts	0.701	0.553	0.4	0.31	0.29	A+C, E	0.20	0.98
		(95% CIs)	(.435 - .856)	(.127 - .810)	(0 - 0.83)	(0 - 0.74)	(0.15 - 0.54)			
		Volume	0.681	0.558	0.15	0.50	0.35	A+C, E	0.19	0.98
		(95% CIs)	(.410 - .842)	(.134 - .812)	(0 - 0.77)	(0 - 0.77)	(0.19 - 0.58)			
		Fractional Anisotropy	0.615	0.089	0.62	0	0.38	AE	1.26	0.26
		(95% CIs)	(.310 - .806)	(-.432 - .573)	(0.07 - 0.80)	(0 - 0)	(0.19 - 0.72)			
		Mean Diffusivity	0.502	0.396	0.69	0	0.31	AE	1.50	0.1
		(95% CIs)	(.155 - .740)	(-.077 - .727)	(0.06 - 0.84)	(0 - 0)	(0.15 - 0.61)			
	Right	Number of Tracts	0.686	0.577	0.08	0.60	0.32	A+C, E	1.87	0.08
		95% CIs	(.411 - .848)	(.161 - .822)	(0 - 0.77)	(0 - 0.79)	(0.17 - 0.54)			
		Volume	0.586	0.486	0	0.59	0.41	A+C,E	1.93	0.07
		95% CIs	(.244 - .799)	(0 - .790)	(0 - 0.71)	(0 - 0.75)	(0.23 - 0.65)			
		Fractional Anisotropy	0.478	0.339	0.36	0.11	0.53	A+C, E	0.32	0.92
		95% CIs	(.098 - .738)	(-.196 - .724)	(0 - 0.70)	(0 - 0.61)	(0.29 - 0.88)			
		Mean Diffusivity	0.655	0.299	0.78	0	0.22	AE	0.75	0.63
		95% CIs	(.355 - .834)	(-.219 - .691)	(0.35 - 0.89)	(0 - 0.34)	(0.10 - 0.46)			
ANTERIOR SEGMENT	Left	Number of Tracts	0.522	0.011	0.46	0	0.54	AE	0.96	0.45
		95% CIs	(.182 - .752)	(-.464 - .487)	(0 - 0.69)	(0 - 0.51)	(0.30 - 0.86)			
		Volume	0.311	0.077	0	0	1	E	1.13	0.34
		95% CIs	(-.082 - .623)	(-.396 - .523)	(0 - 0.61)	(0 - 0.43)	(0.38 - 1)			
		Fractional Anisotropy	0.380	0.085	0	0	1	E	0.86	0.54
		95% CIs	(.005 - .663)	(-.403 - .541)	(0 - 0.64)	(0 - 0)	(0.35 - 1)			
		Mean Diffusivity	0.527	0.083	0.54	0	0.46	A+C,E	0.97	0.44
		95% CIs	(.188 - .755)	(-.390 - .528)	(0 - 0.77)	(0 - 0.36)	(0.22 - 0.87)			
	Right	Number of Tracts	0.491	0.490	0.05	0.45	0.50	A+C, E	2.01	0.06
		95% CIs	(.124 - .741)	(.040 - .778)	(0 - 0.70)	(0 - 0.68)	(0.27 - 0.78)			
		Volume	0.502	0.501	0	0.49	0.51	A+C, E	0.86	0.52
		95% CIs	(.129 - .752)	(.038 - .790)	(0 - 0.68)	(0 - 0.69)	(0.28 - 0.77)			
		Fractional Anisotropy	0.638	0.231	0.66	0	0.34	AE	0.99	0.43
		95% CIs	(.345 - .819)	(-.270 - .639)	(0.09 - 0.82)	(0 - 0.42)	(0.17 - 0.64)			
		Mean Diffusivity	0.722	0.485	0.70	0.03	0.27	AE	0.40	0.9
		95% CIs	(.475 - .864)	(.017 - .782)	(0.01 - 0.85)	(0 - 0.60)	(0.14 - 0.51)			
POSTERIOR SEGMENT	Left	Number of Tracts	0.567	0.262	0.48	0.06	0.46	A+C, E	0.54	0.77
		95% CIs	(.218 - .789)	(-.276 - .681)	(0 - 0.75)	(0 - 0.66)	(0.24 - 0.78)			
		Volume	0.469	-0.066	0	0	1	E	1.04	0.4
		95% CIs	(.076 - .737)	(-.521 - .426)	(0 - 0.67)	(0 - 0.45)	(0.32 - 0.99)			
		Fractional Anisotropy	0.643	0.197	0.67	0	0.33	AE	1.04	0.22
		95% CIs	(.352 - .821)	(-.288 - .607)	(0.13 - 0.83)	(0 - 0)	(0.17 - 0.62)			
		Mean Diffusivity	0.595	0.268	0.68	0	0.32	AE	0.83	0.56
		95% CIs	(.282 - .794)	(-.218 - .652)	(0.07 - 0.83)	(0 - 0.45)	(0.16 - 0.61)			
	Right	Number of Tracts	0.353	0.046	0	0	1	E	1.08	0.37
		95% CIs	(-.112 - .694)	(-.484 - .561)	(0 - 0.58)	(0 - 0.54)	(0.41 - 1)			
		Volume	0.449	0.233	0.05	0.34	0.61	A+C, E	1.66	0.13
		95% CIs	(.041 - .731)	(-.253 - .630)	(0 - 0.64)	(0 - 0.62)	(0.35 - 0.91)			
		Fractional Anisotropy	0.391	0.198	0	0	1	E	1.14	0.33
		95% CIs	(.017 - .670)	(-.318 - .630)	(0 - 0.62)	(0 - 0)	(0.37 - 0.98)			
		Mean Diffusivity	0.705	0.273	0.78	0	0.22	AE	0.53	0.81
		95% CIs	(.448 - .856)	(-.212 - .655)	(0.38 - 0.88)	(0 - 0)	(0.11 - 0.46)			

Appendix B

Chapter 6.
Tables and figures of appendix B:

Table 6.3.1 Group statistics of the behavioural measures; 1 - controls, 2 - ASD group.

Group Statistics

	group	N	Mean	Std. Deviation	Std. Error Mean
Verbal_IQ	1.00	61	110.1639	12.77652	1.63587
	2.00	61	110.5738	12.77557	1.63574
Full_IQ	1.00	61	114.5410	11.59824	1.48500
	2.00	61	111.5738	12.24263	1.56751
FAS	1.00	60	43.2000	12.54119	1.61906
	2.00	60	38.4667	13.17865	1.70136
NWR	1.00	59	22.7797	3.54803	.46191
	2.00	60	21.7167	3.85804	.49807
AQ	1.00	61	14.0656	6.41578	.82146
	2.00	59	30.3898	8.33153	1.08467
@EQ	1.00	60	44.1333	11.79553	1.52280
	2.00	59	22.6102	11.65305	1.51710
COMMUNICATION	1.00	61	1.7377	1.67234	.21412
	2.00	59	6.3051	2.51378	.32727
SOCIAL	1.00	61	1.7541	1.97193	.25248
	2.00	59	6.0847	2.70560	.35224
AGE	1.00	61	27.6885	6.41493	.82135
	2.00	61	26.0492	7.16107	.91688

Table 6.3.2 Group comparison on behavioural measures; 1 - controls, 2 - ASD group.

Independent Samples Test

		t-test for Equality of Means		
		t	df	Sig. (2-tailed)
Verbal_IQ	Equal variances assumed	-.177	120	.860
	Equal variances not assumed	-.177	120.000	.860
Full_IQ	Equal variances assumed	1.374	120	.172
	Equal variances not assumed	1.374	119.651	.172
FAS	Equal variances assumed	2.015	118	.046
	Equal variances not assumed	2.015	117.711	.046
NWR	Equal variances assumed	1.564	117	.121
	Equal variances not assumed	1.565	116.482	.120
AQ	Equal variances assumed	-12.049	118	.000
	Equal variances not assumed	-11.998	108.962	.000
@EQ	Equal variances assumed	10.012	117	.000
	Equal variances not assumed	10.013	116.997	.000
COMMUNICATION	Equal variances assumed	-11.755	118	.000
	Equal variances not assumed	-11.679	100.485	.000
SOCIAL	Equal variances assumed	-10.044	118	.000
	Equal variances not assumed	-9.993	105.889	.000
AGE	Equal variances assumed	1.332	120	.185
	Equal variances not assumed	1.332	118.576	.185

Table 6.3.3 Descriptives between the three groups on behavioural measures; 1 - Asperger group, 2 - High functioning autism group, 3 - Controls; significant at 0.05 level.

Descriptives

			N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
							Lower Bound	Upper Bound
Verbal_IQ	1.00		37	110.3514	13.61416	2.23815	105.8122	114.8905
	2.00		24	110.9167	11.63920	2.37584	106.0019	115.8315
	3.00		61	110.1639	12.77652	1.63587	106.8917	113.4362
	Total		122	110.3689	12.72481	1.15205	108.0881	112.6496
	Model	Fixed Effects			12.82809	1.16140	108.0692	112.6685
		Random Effects				1.16140[a]	105.3717[a]	115.3660[a]
Full_IQ	1.00		37	111.9459	12.54314	2.06208	107.7639	116.1280
	2.00		24	111.0000	12.00724	2.45097	105.9298	116.0702
	3.00		61	114.5410	11.59824	1.48500	111.5705	117.5114
	Total		122	113.0574	11.96848	1.08358	110.9122	115.2026
	Model	Fixed Effects			11.97021	1.08373	110.9115	115.2033
		Random Effects				1.08373[a]	108.3945[a]	117.7203[a]
FAS	1.00		36	38.2500	14.31558	2.38593	33.4063	43.0937
	2.00		24	38.7917	11.55320	2.35829	33.9132	43.6702
	3.00		60	43.2000	12.54119	1.61906	39.9603	46.4397
	Total		120	40.8333	13.02830	1.18932	38.4784	43.1883
	Model	Fixed Effects			12.91733	1.17919	38.4980	43.1686
		Random Effects				1.77216	33.2084	48.4583
NWR	1.00		36	21.3056	3.76313	.62719	20.0323	22.5788
	2.00		24	22.3333	3.99638	.81576	20.6458	24.0209

Descriptives

							95% Confidence Interval for Mean	
			N	Mean	Std. Deviation	Std. Error	Lower Bound	Upper Bound
	1.00		37	110.3514	13.61416	2.23815	105.8122	114.8905
	2.00		24	110.9167	11.63920	2.37584	106.0019	115.8315
	3.00		59	22.7797	3.54803	.46191	21.8550	23.7043
	Total		119	22.2437	3.73024	.34195	21.5665	22.9209
	Model	Fixed Effects			3.70590	.33972	21.5708	22.9166
		Random Effects				.47380	20.2051	24.2823
AQ	1.00		37	30.6757	7.88830	1.29683	28.0456	33.3058
	2.00		22	29.9091	9.20098	1.96166	25.8296	33.9886
	3.00		61	14.0656	6.41578	.82146	12.4224	15.7087
	Total		120	22.0917	11.03394	1.00726	20.0972	24.0861
	Model	Fixed Effects			7.44648	.67977	20.7454	23.4379
		Random Effects				6.47943	-5.7871	49.9704
@EQ	1.00		37	21.8649	12.38539	2.03615	17.7354	25.9944
	2.00		22	23.8636	10.46216	2.23054	19.2250	28.5023
	3.00		60	44.1333	11.79553	1.52280	41.0862	47.1804
	Total		119	33.4622	15.90906	1.45838	30.5742	36.3502
	Model	Fixed Effects			11.75535	1.07761	31.3278	35.5965
		Random Effects				8.51550	-3.1771	70.1014
COMMUNICATION	1.00		37	6.3784	2.39619	.39393	5.5794	7.1773
	2.00		22	6.1818	2.75398	.58715	4.9608	7.4029
	3.00		61	1.7377	1.67234	.21412	1.3094	2.1660
	Total		120	3.9833	3.12212	.28501	3.4190	4.5477
	Model	Fixed Effects			2.13593	.19498	3.5972	4.3695
		Random Effects				1.81261	-3.8157	11.7823
SOCIAL	1.00		37	6.3243	2.71880	.44697	5.4178	7.2308

Descriptives

			N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
							Lower Bound	Upper Bound
	1.00		37	110.3514	13.61416	2.23815	105.8122	114.8905
	2.00		24	110.9167	11.63920	2.37584	106.0019	115.8315
	2.00		22	5.6818	2.69720	.57505	4.4859	6.8777
	3.00		61	1.7541	1.97193	.25248	1.2491	2.2591
	Total		120	3.8833	3.20237	.29234	3.3045	4.4622
	Model	Fixed Effects			2.36099	.21553	3.4565	4.3102
		Random Effects				1.72569	-3.5417	11.3084
AGE	1.00		37	24.9459	7.23013	1.18863	22.5353	27.3566
	2.00		24	27.7500	6.85407	1.39908	24.8558	30.6442
	3.00		61	27.6885	6.41493	.82135	26.0456	29.3315
	Total		122	26.8689	6.81994	.61745	25.6465	28.0913
	Model	Fixed Effects			6.75593	.61165	25.6577	28.0800
		Random Effects				.95071	22.7783	30.9594

Table 6.3.4 ANOVA analysis between the three groups on behavioural measures; 1 - Asperger group, 2 - High functioning autism group, 3 - Controls; significant at 0.05 level.

Multiple Comparisons

Bonferroni

Dependent Variable	(I) GROUPS	(J) GROUPS	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
Verbal_IQ	1.00	2.00	-.56532	3.36218	1.000	-8.7297	7.5990
		3.00	.18742	2.67306	1.000	-6.3036	6.6784
	2.00	1.00	.56532	3.36218	1.000	-7.5990	8.7297
		3.00	.75273	3.09101	1.000	-6.7532	8.2586
	3.00	1.00	-.18742	2.67306	1.000	-6.6784	6.3036
		2.00	-.75273	3.09101	1.000	-8.2586	6.7532
Full_IQ	1.00	2.00	.94595	3.13733	1.000	-6.6724	8.5643
		3.00	-2.59504	2.49430	.901	-8.6519	3.4619
	2.00	1.00	-.94595	3.13733	1.000	-8.5643	6.6724
		3.00	-3.54098	2.88430	.666	-10.5449	3.4630
	3.00	1.00	2.59504	2.49430	.901	-3.4619	8.6519
		2.00	3.54098	2.88430	.666	-3.4630	10.5449
FAS	1.00	2.00	-.54167	3.40401	1.000	-8.8096	7.7263
		3.00	-4.95000	2.72321	.215	-11.5644	1.6644
	2.00	1.00	.54167	3.40401	1.000	-7.7263	8.8096
		3.00	-4.40833	3.11983	.481	-11.9861	3.1694
	3.00	1.00	4.95000	2.72321	.215	-1.6644	11.5644
		2.00	4.40833	3.11983	.481	-3.1694	11.9861

Multiple Comparisons

Bonferroni

Dependent Variable	(I) GROUPS	(J) GROUPS	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
		2.00	-.56532	3.36218	1.000	-8.7297	7.5990
NWR	1.00	2.00	-1.02778	.97659	.884	-3.4001	1.3445
		3.00	-1.47411	.783 75	.188	-3.3780	.4298
	2.00	1.00	1.02778	.976 59	.884	-1.3445	3.4001
		3.00	-.44633	.897 22	1.000	-2.6259	1.7332
	3.00	1.00	1.47411	.783 75	.188	-.4298	3.3780
		2.00	.44633	.897 22	1.000	-1.7332	2.6259
AQ	1.00	2.00	.76658	2.00477	1.000	-4.1028	5.6360
		3.00	16.61010*	1.55 167	.000	12.8413	20.3789
	2.00	1.00	-.76658	2.00 477	1.000	-5.6360	4.1028
		3.00	15.84352*	1.85 189	.000	11.3455	20.3415
	3.00	1.00	-16.61010*	1.55 167	.000	-20.3789	-12.8413
		2.00	-15.84352*	1.85 189	.000	-20.3415	-11.3455
@EQ	1.00	2.00	-1.99877	3.16482	1.000	-9.6867	5.6892
		3.00	-22.26847*	2.45 723	.000	-28.2375	-16.2994
	2.00	1.00	1.99877	3.16 482	1.000	-5.6892	9.6867
		3.00	-20.26970*	2.92 992	.000	-27.3870	-13.1524
	3.00	1.00	22.26847*	2.45 723	.000	16.2994	28.2375
		2.00	20.26970*	2.92 992	.000	13.1524	27.3870

Multiple Comparisons

Bonferroni

Dependent Variable	(I) GROUPS	(J) GROUPS	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
		2.00	-.56532	3.36218	1.000	-8.7297	7.5990
COMMUNICATION	1.00	2.00	.19656	.57504	1.000	-1.2002	1.5933
		3.00	4.64067*	.44508	.000	3.5596	5.7217
	2.00	1.00	-.19656	.57504	1.000	-1.5933	1.2002
		3.00	4.44411*	.53119	.000	3.1539	5.7343
	3.00	1.00	-4.64067*	.44508	.000	-5.7217	-3.5596
		2.00	-4.44411*	.53119	.000	-5.7343	-3.1539
SOCIAL	1.00	2.00	.64251	.63564	.943	-.9014	2.1864
		3.00	4.57023*	.49197	.000	3.3753	5.7652
	2.00	1.00	-.64251	.63564	.943	-2.1864	.9014
		3.00	3.92772*	.58716	.000	2.5016	5.3539
	3.00	1.00	-4.57023*	.49197	.000	-5.7652	-3.3753
		2.00	-3.92772*	.58716	.000	-5.3539	-2.5016
AGE	1.00	2.00	-2.80405	1.77070	.348	-7.1038	1.4957
		3.00	-2.74258	1.40777	.161	-6.1611	.6759
	2.00	1.00	2.80405	1.77070	.348	-1.4957	7.1038
		3.00	.06148	1.62789	1.000	-3.8915	4.0145
	3.00	1.00	2.74258	1.40777	.161	-.6759	6.1611
		2.00	-.06148	1.62789	1.000	-4.0145	3.8915

*. The mean difference is significant at the 0.05 level.

Table 6.3.5 Regression analysis for the long segment in the left hemisphere; showing significant differences in diffusion parameters at $p < 0.05$; all the measures were co-varied for centre; FA measures co-varied additionally for age; number of tracts and volume co-varied additionally for total number of streamlines or total brain volume.

Dependent Variable	Index	N	Mean	Std. Deviation	Sig.
Long Left Segment FA	**Controls**	**60**	0.50421	.0201	**0.047**
	Patients	**60**	0.49608	.0230	
Long Left Segment MD	**Controls**	**60**	0.000741	0.000023	**0.002**
	Patients	**60**	0.000755	0.000025	
Long Left Segment Dperp	**Controls**	**60**	0.000513	0.000026	**0.003**
	Patients	**60**	0.000528	0.000028	
Long Left Segment Volume	**Controls**	**60**	16.081	4.183	0.098
	Patients	**60**	14.775	4.330	
Long Left Segment No of Tracts	**Controls**	**60**	292.18	108.3	**0.039**
	Patients	**60**	250.8	104.9	

Table 6.3.6 Regression analysis for the anterior segment in the left hemisphere; showing significant differences in diffusion parameters at $p < 0.05$; all the measures were co-varied for centre; FA measures co-varied additionally for age; number of tracts and volume co-varied additionally for total number of streamlines or total brain volume.

Dependent Variable	Index	N	Mean	Std. Deviation	Sig.
Anterior Left Segment No of Tracts	**Controls**	**61**	132.52	78.50	**0.031**
	Patients	**60**	103.1	62.81	
Anterior Left Segment Dperp	**Controls**	**61**	0.000554	0.000030	**0.003**
	Patients	**60**	0.000572	0.000036	
Anterior Left Segment MD	**Controls**	**61**	0.000758	0.000026	**0.003**
	Patients	**60**	0.000774	0.000031	
Anterior Left Segment FA	**Controls**	**61**	0.459	0.0266	**0.025**
	Patients	**60**	0.448	0.0307	
Anterior Left Segment Volume	**Controls**	**61**	8.491	3.722	**0.025**
	Patients	**60**	7.010	2.971	

6.3.7 Regression analysis for the posterior left segment; showing significant differences in diffusion parameters at $p < 0.05$; all the measures were co-varied for centre; FA measures co-varied additionally for age; number of tracts and volume co-varied additionally for total number of streamlines or total brain volume.

Dependent Variable	Index	N	Mean	Std. Deviation	Sig. (centre)
Posterior Left Segment MD	**Controls**	**61**	0.000752	0.000022	**0.007**
	Patients	**61**	0.000765	0.000030	
Posterior Left Segment FA	**Controls**	**61**	0.454	0.0264	0.883
	Patients	**61**	0.453	0.0236	
Posterior Left Segment Dperp	**Controls**	**61**	0.000549	0.0000256	0.060
	Patients	**61**	0.000558	0.0000301	
Posterior Left Segment Volume	**Controls**	**61**	9.217	2.827	0.383
	Patients	**61**	9.77	4.08	
Posterior Left Segment No Tracts	**Controls**	**61**	156.70	67.72	0.353
	Patients	**61**	170.5	94.26	

Table 6.3.8 Regression analysis for the long segment in the right hemisphere; showing significant differences in diffusion parameters at $p < 0.05$; all the measures were co-varied for centre; FA measures co-varied additionally for age; number of tracts and volume co-varied additionally for total number of streamlines or total brain volume.

Dependent Variable	Index	N	Mean	Std. Deviation	Sig.
Long Right Segment No of Tracts	**Controls**	**57**	161.75	114.114	0.072
	Patients	**56**	127.12	86.298	
Long Right Segment Volume	**Controls**	**57**	10.30	4.730	0.264
	Patients	**56**	9.37	4.064	
Long Right Segment MD	**Controls**	**57**	0.000744	0.000020	0.162
	Patients	**56**	0.000751	0.000026	
Long Right Segment FA	**Controls**	**57**	0.491	0.0243	0.210
	Patients	**56**	0.484	0.0254	
Long Right Segment Dperp	**Controls**	**57**	0.000521	0.0000239	0.094
	Patients	**56**	0.000529	0.0000266	

Table 6.3.9 Regression analysis for the anterior segment in the right hemisphere; showing significant differences in diffusion parameters at $p < 0.05$; all the measures were co-varied for centre; FA measures co-varied additionally for age; number of tracts and volume co-varied additionally for total number of streamlines or total brain volume.

Dependent Variable	Index	N	Mean	Std. Deviation	Sig.
Anterior Right Segment No of Tracts	**Controls**	**61**	210.11	125.08	0.428
	Patients	**61**	192.59	119.91	
Anterior Right Segment Volume	**Controls**	**61**	11.732	4.939	0.603
	Patients	**61**	11.267	4.989	
Anterior Right Segment FA	**Controls**	**61**	0.470	0.027	0.188
	Patients	**61**	0.464	0.024	
Anterior Right Segment MD	**Controls**	**61**	0.000762	0.0000239	0.085
	Patients	**61**	0.000770	0.0000266	
Anterior Right Segment Dperp	**Controls**	**61**	0.00055	0.000027	0.080
	Patients	**61**	0.000559	0.0000283	

Table 6.3.10 Regression analysis for the posterior segment in the right hemisphere; showing significant differences in diffusion parameters at $p < 0.05$; all the measures were co-varied for centre; FA measures co-varied additionally for age; number of tracts and volume co-varied additionally for total number of streamlines or total brain volume.

Dependent Variable	Index	N	Mean	Std. Deviation	Sig.
Posterior Right Segment MD	**Controls**	**60**	0.000761	0.000025	**0.005**
	Patients	**59**	0.000775	0.000031	
Posterior Right Segment Dperp	**Controls**	**60**	0.000556	0.000027	**0.046**
	Patients	**59**	0.000567	0.000031	
Posterior Right Segment FA	**Controls**	**60**	0.452	0.0284	0.876
	Patients	**59**	0.437	0.0267	
Posterior Right Segment Volume	**Controls**	**60**	7.23	2.85	0.829
	Patients	**59**	7.35	3.19	
Posterior Right Segment No Tracts	**Controls**	**60**	134.32	73.69	0.945
	Patients	**59**	133.33	82.68	

Table 6.3.11 Significant positive correlations between diffusion measures of the three segments and AQ Communication scores

Dependent Variable	Dependent Variable	N	r	Sig.
Long Left Segment	**MD**	**120**	.200	**.030**
	Dperp		.219	**.017**
Anterior Left Segment	**MD**	**121**	.224	**.014**
	Dperp		.213	**.020**
Posterior Left Segment	**MD**	**122**	.211	**.021**

Table 6.3.12 Significant positive correlations between diffusion measures of the anterior and posterior left segments and AQ Social scores

Dependent Variable	Dependent Variable	N	r	Sig.
Anterior Left Segment	**MD**	**121**	.182	**.048**
Posterior Left Segment	**MD**	**122**	.189	**.038**

Table 6.3.13 Significant negative correlations between diffusion measures of the three segments and EQ Empathy scores

Dependent Variable	Dependent Variable	N	r	Sig.
Long Left Segment	**MD**	**120**	-.221	**.017**
	Dperp		-.189	**.041**
Anterior Left Segment	**MD**	**121**	-.228	**.013**
	Dperp		-.220	**.017**
Posterior Left Segment	**MD**	**122**	-.208	**.023**
Posterior Right Segment	**MD**	**122**	-.185	**.045**

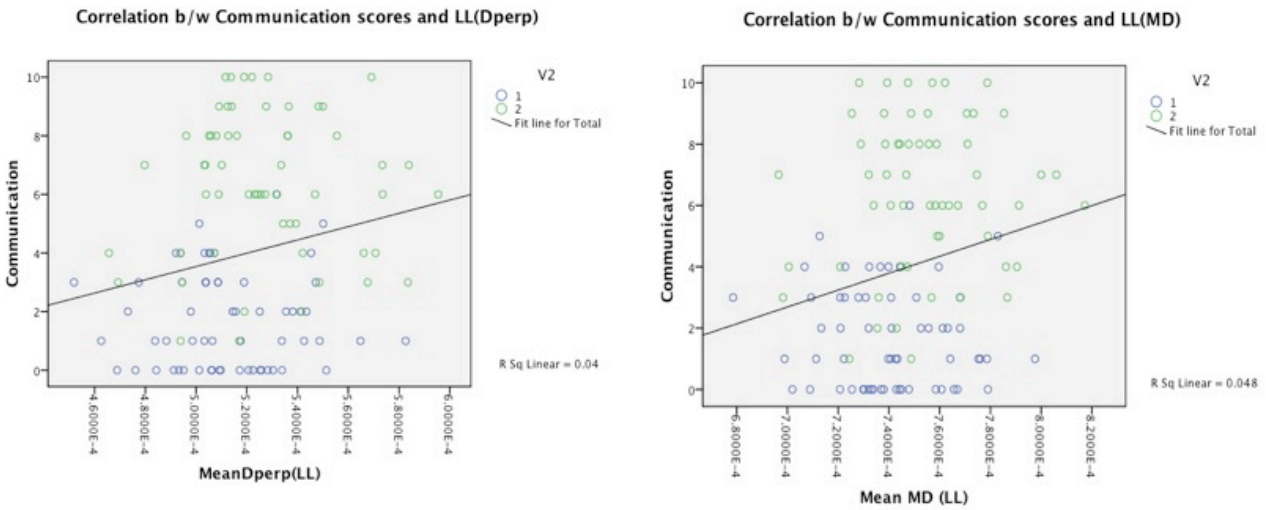

Fig 6.3.14 Significant positive correlations between AQ Communication scores and mean diffusivity (MD) (r=.200, P=.030) and Dperp (r=.219, P=.017) of the LL (left long) segment

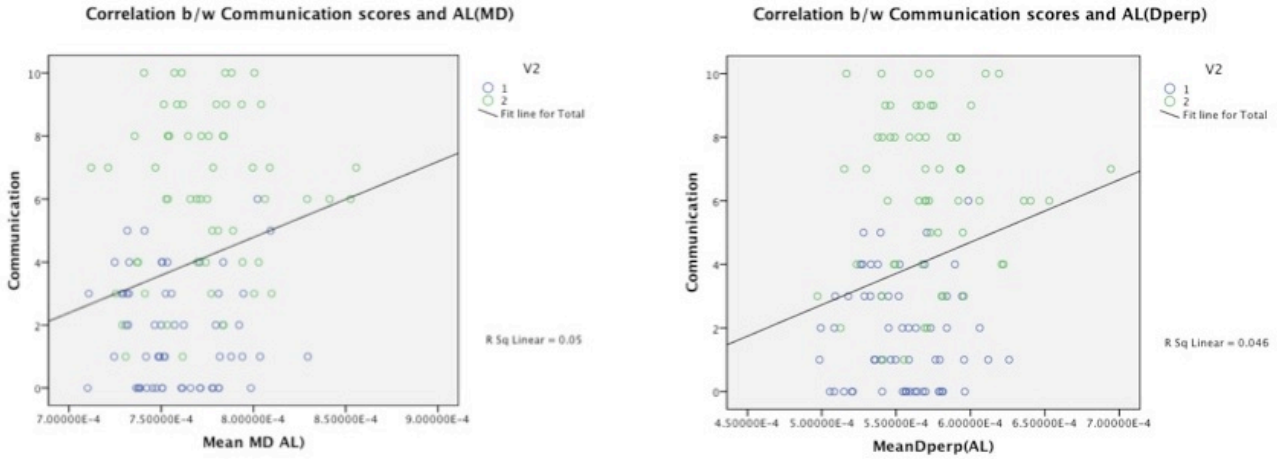

Fig 6.3.15 Significant positive correlations between AQ Communication scores and mean diffusivity (MD) (r=.224, P=.014) and Dperp (r=.213, P=.020) of the AL (left anterior) segment

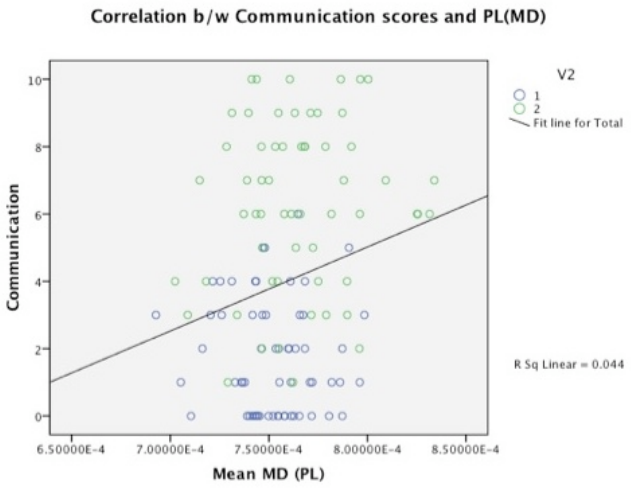

Fig 6.3.16 Significant positive correlation between AQ Communication scores and mean diffusivity (MD) (r=.211, P=.021) of the PL (left posterior) segment

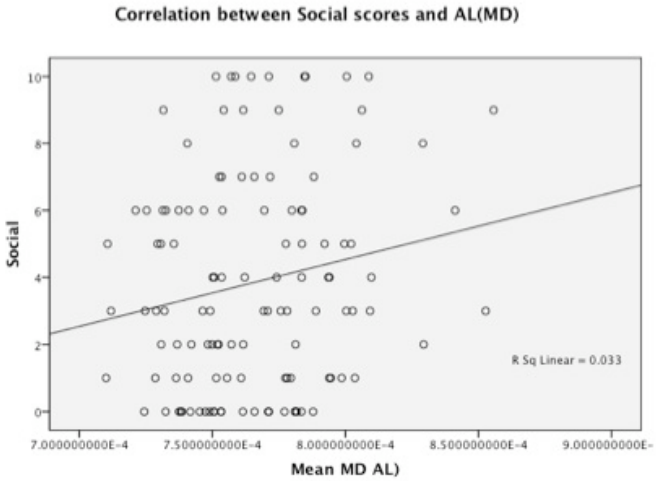

Fig 6.3.17 Significant positive correlation between AQ Social scores and mean diffusivity (MD) (r=.182, P=.048) of the AL (left anterior) segment

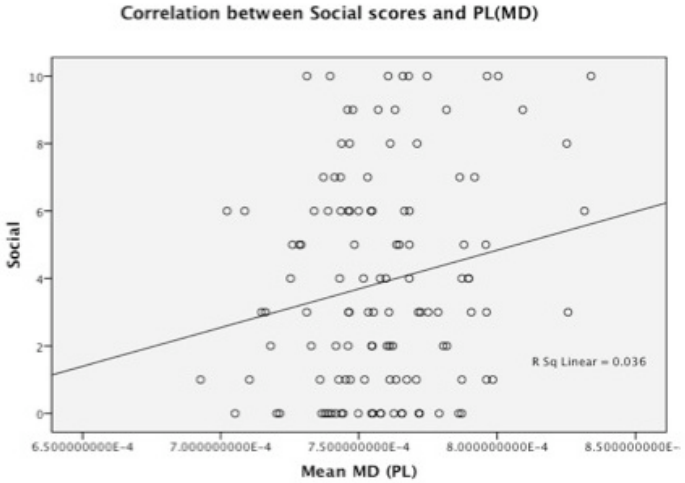

Fig 6.3.18 Significant positive correlation between AQ Social scores and mean diffusivity (MD) (r=.189, P=.038) of the PL (left posterior) segment

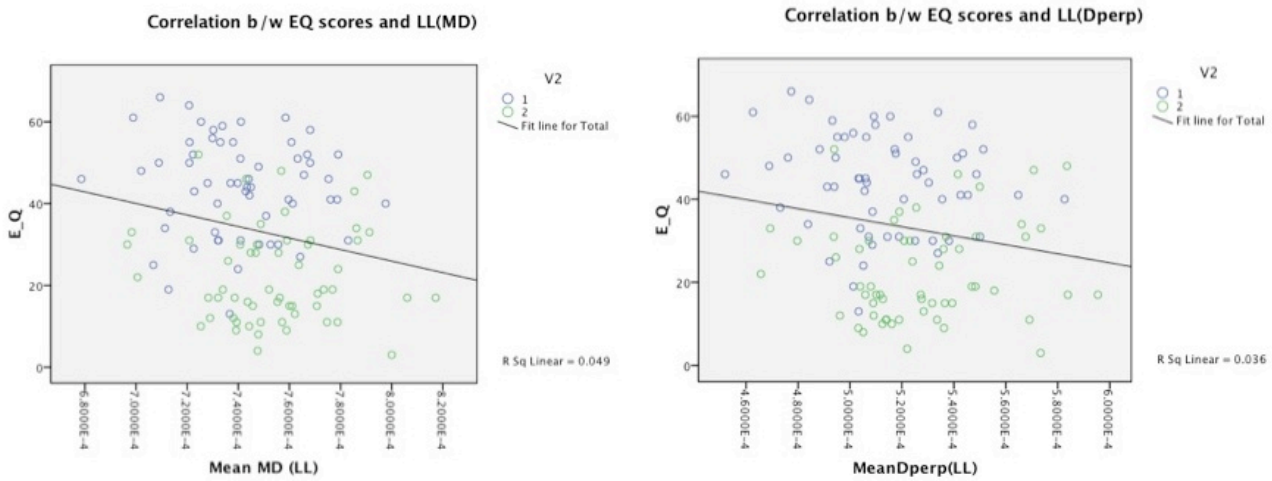

Fig 6.3.19 Significant negative correlations between EQ (empathy) scores and mean diffusivity (MD) (r=-.221, P=.017) and Dperp (r=-.189, P=.041) of the LL (left long) segment

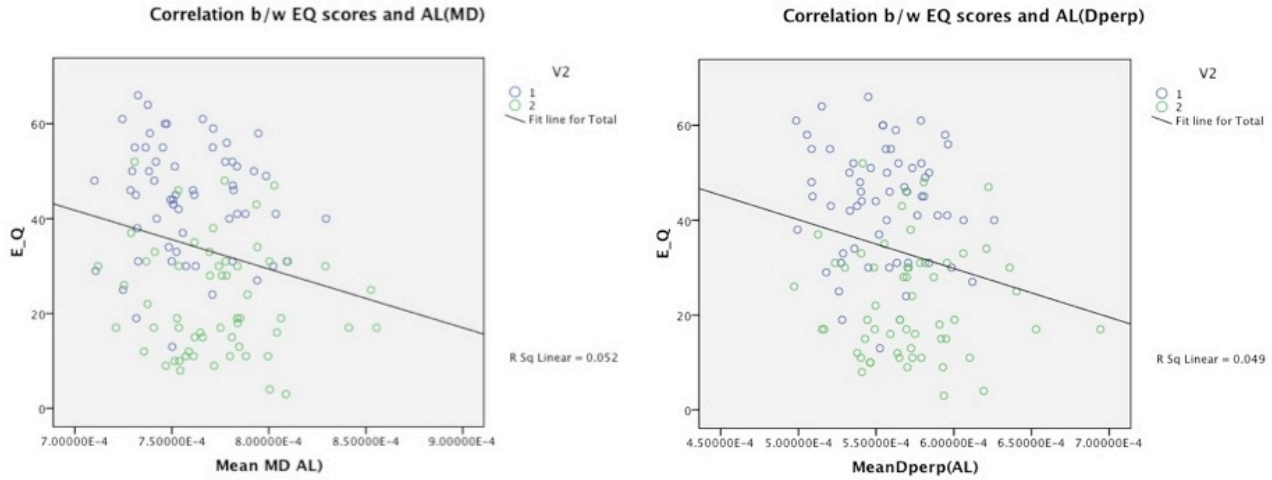

Fig 6.3.20 Significant negative correlations between EQ (empathy) scores and mean diffusivity (MD) (r=-.228, P=.013) and Dperp (r=-.220, P=.017) of the AL (left anterior) segment

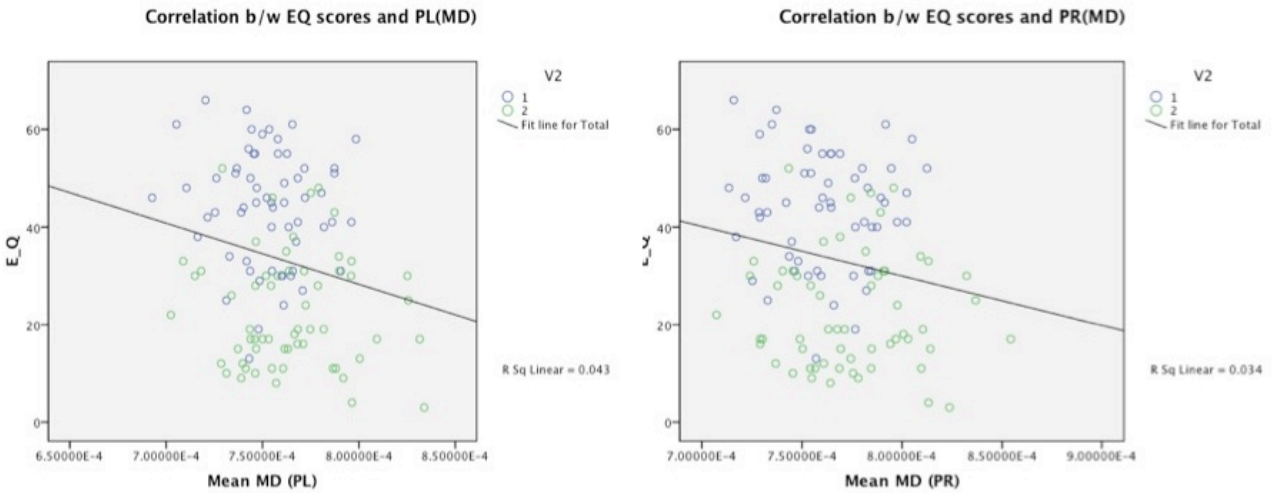

Fig 6.3.21 Significant negative correlations between EQ (empathy) scores and left mean diffusivity (MD) (r=-.208, P=.023) (left table) and right MD (r=-.185, P=.045) (right table) of the posterior segment

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